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13. ABSTRACT (Maximum 200 Words)

This project aims to collect NF1 patient DNAs required to identify neurofibroma burden modifier genes, to perform an allele association study for three classes of potential modifiers, and to evaluate more global approaches. Over four years we aim to collect 1200 DNAs from adult NF1 patients that represent the top and bottom 20% of dermal neurofibroma burden in various age cohorts. We will use these DNAs in a case-control allele association study, to test whether neurofibroma numerical variability reflects (1) allelic differences in genes that maintain genome stability; (2) differences in the *NF1* gene itself or in closely linked genes; or (3) differences in genes involved in signaling between neurofibroma constituent cells. The Army Office of Regulatory Compliance took until February 2004 to sign an approval memorandum for this study and disallowed one of our major proposed routes of patient recruitment. Our major progress during the first year, in addition to obtaining regulatory approval, has been the implementation of a relational database of 990 potential modifier genes in the signaling category. Other effort has gone towards enrolling additional collaborators to make up for the disallowed recruitment route, and towards implementing more efficient methods of SNP genotyping.

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Introduction

Neurofibromatosis type 1 (NF1) affects approximately 2-3 in 10,000 worldwide. A high degree of unpredictability and variability of symptoms is among the hallmarks of NF1. This variable expressivity tends to increase patient anxiety and is a serious problem for clinical trials. Thus, significant resources have been devoted to better describing the natural history of NF1, apparently without much to show for the effort. We have chosen an alternative approach to increase the predictability of NF1, based on studies that have implicated symptom-specific modifier genes as important determinants of the clinical variability in NF1 [1-4]. Our specific aims are to collect somatic DNAs from 1200 NF1 patients that represent the top and bottom 20% of dermal neurofibroma burden, and to perform a case-control allele association study to evaluate three classes of potential neurofibroma burden modifier genes.

Body

Our Statement of Work for year 1 listed four goals: (1) to assemble a 500-600 member case-control patient DNA panel; (2) to identify and prioritize SNPs in all classes of potential modifiers; (3) to design and validate SNP genotyping assays; and (4) to begin genotyping of >0.1 VAF SNPs in the 600 member exploration panel.

Progress on Aim 1: We had not listed obtaining regulatory approval as a separate goal for year 1, because we did not anticipate that it would take over 8 months to get approval. We failed to anticipate this delay because the Army Regulatory Compliance Office had previously approved our very similar smaller scale pilot study, funded as an Idea Award in 1999. Thus, we did not anticipate that it would take until February of 2004 to obtain approval, nor did we expect that significant changes in recruitment procedures/study design would be mandated. The regulatory problems arose in part because the Army Office did not agree to new HIPAA-mandated language in our MGH/Partners Healthcare-approved consent form. We did not have the authority to alter this language, and a conference call between all concerned authorities did not resolve this issue. Another significant problem was that a previously approved method of recruiting patients who contacted us after learning about the study, for example in patient organization newsletters, was no longer deemed allowable. In the end the only solution to both problems was our agreement to stop recruiting patients using our own consent form, and to henceforth restrict our analysis to anonymous DNA samples provided to us by collaborators. In the MGH NF clinic, DNA samples are currently collected under Dr. James Gusella's protocol, which allows sample sharing with other investigators. Of our other proposed collaborators, several are in the process of amending their protocols so that sharing of anonymous samples is also explicitly allowed, and Dr. Rosalie Ferner (Guy's Hospital, London, UK) has just obtained local approval. Unfortunately, Dr. Felix Mautner (Klinik Ochsenzoll, Hamburg, Germany) who had previously agreed to supply up to 300 new patient samples in addition to 288 DNA samples he had available, has indicated his unwillingness to further collaborate without receiving significant monetary compensation. Moreover, when Dr. Mautner did provide the 288 available patient DNA samples, it turned out that 116 patients did not meet our eligibility criteria. Moreover, among the 172 eligible samples, 51 contained no detectable DNA. While it may be possible to analyze at least some of these latter samples after whole genome amplification, this experience has re-stimulated our efforts to find

additional foreign or domestic collaborators. After presenting the design and goals of this study as a platform presentation at the 2003 annual NNFF consortium meeting and as a poster at the 2004 meeting, several potential new collaborators have expressed interest in helping us achieve our recruitment goals. We are particularly interested in a proposal by Dr. Susan Huson to serve as our study coordinator in Great Britain. Dr. Huson is the author of several comprehensive clinical studies and monographs of NF1 [5], and has had a long interest in the role of modifier genes. At the just concluded 2004 NNFF consortium meeting, she proposed to serve as our study coordinator to assist with patient recruitment from several UK clinics. We have asked Dr. Huson to put together a proposal so that we can evaluate what would be required. Interest in participating in this study has also been expressed by Dr. Dusica Babovic of the Scripps Clinic, by Dr. Margaret Wallace of the University of Florida, and by Drs. Priscilla Short of the University of Chicago.

As a result of Dr. Mautner's unexpected decision, because many of his promised samples were either not eligible or not useable, and because we are no longer allowed to recruit patients, we are experiencing some delay in patient recruitment. We have been in discussions with Dr. Bruce Korf (U. Alabama, Birmingham), who encountered similar challenges in his NF1 natural history study. Dr. Korf is convening a U.S. Army NF Research Program sponsored meeting in September to discuss the specifications of a neurofibromatosis clinical research operations center. Such a center would facilitate and coordinate clinical research projects on neurofibromatosis, providing a service to PI's to facilitate participant recruitment. We strongly support the creation of such a center, which may come late for this project, but which would be essential for any future study to identify genetic modifiers for less common NF1 symptoms.

Progress on aim 2: In our proposal we described the design of relational databases to collect and manage information on potential hypomorphic alleles among a comprehensive set of genome stability genes. We have continued this bioinformatics/data mining effort and have identified 964 missense SNPs among 319 putative genome stability genes. Among these SNPs, 176 have a minor allele frequency of >4%. This compares with 576 missense SNPs (110 >4%) in 244 candidate modifier genes that we had identified when this proposal was written. As planned, we also identified a comprehensive set of VNTRs and SNPs in the 1 MB segment deleted in patients

with microdeletions [6]. Also as planned, we set out to identify candidate genes involved in signal transduction between the cell types that make up neurofibromas. This work is made easier by the existence of comprehensive surveys of several classes of human signaling proteins [7,8]. We have expanded this effort by constructing a new set of databases to collect information on human members of the Ras GTPase superfamily, their regulators and interactors/effectors. Using the same cross-species BLAST approach we previously used to identify structural relatives of Ras superfamily GTPase Activating Proteins (Bernards A. GAPs Galore! A survey of putative Ras superfamily GTPase activating proteins in man and Drosophila. Biochim. Biophys. Acta 2003. 1603:47-82; Appendix 1), we so far have identified 170 Ras superfamily members, 159 potential and confirmed Ras superfamily GAPs, 159 potential and confirmed exchange factors, and 294 effectors/interacting proteins plus 242 paralogs, for a total of 1024 human Ras superfamily signal transduction genes.

Among the 319 genes in the genome stability category, we assigned three priorities. Thus, priority 1 genes are those with known hypomorphic or cancer-implicated alleles, genes known to be haploinsufficient, or any gene with non-conservative missense substitutions. Priority 2 genes are genes that are functionally related to cancer-implicated genes, whereas the remaining genes are assigned priority 3. Similarly, we have used a different set of criteria to assign priorities among members of the signal transduction class of potential modifiers. The identification of missense SNPs among candidate modifiers previously required great effort, but is made much easier by vastly improved online SNP databases. Based on our previous experience we expect to find ~500 >4% allele frequency missense SNPs, about 1/4 to 1/3 of which may affect priority 1 genes. However, we are still adding additional signaling proteins to our database, so the total number of to-be-genotyped common missense SNPs in this category may eventually reach ~300.

Progress on aim 3: Beyond much effort spend on data mining, we have used the delay in patient recruitment to gain further experience with high throughput SNP genotyping and haplotype analysis. In a project funded by the Avon Corporation, we used various methods to genotype multiple genome stability missense SNPs in an available panel of >600 early onset breast cancer patients and >450 controls. This work identified highly significant associations ($P<0.001$) for several FANCA missense alleles that are in strong linkage disequilibrium. We generated

complete genotypes for 12 SNPs in a sub-group of patients and controls, and collaborated with Dr. Mark Daly of the Whitehead/MIT Genome Center to identify a disease-associated haplotype. We also renewed our collaboration with Dr. Stacey Gabriel (previously of the MIT Genome Center), who currently is Director of the Genetic Analysis Platform at the Broad Institute of Harvard and MIT. With Dr. Gabriel we are exploring the use of the currently favored Illumina™ SNP genotyping platform. Thus, we remain confident that we will be able to identify and analyze a comprehensive set of candidate modifier SNPs in a timely and cost-effective manner once the minimum number of patients required for statistical significance has been recruited.

Progress on aim 4: Validated genotyping assays have been designed and tested for many SNPs, but we have postponed genotyping until the number of DNA samples required for statistical significance is available.

Key Research Accomplishments:

- Identified 964 missense SNPs among 319 potential modifier genes in the genome stability category.
- Prioritized genes and SNPs to be analyzed.
- Identified a comprehensive set of VNTRs and missense SNPs in the 11 genes commonly deleted in NF1 microdeletion patients.
- Designed and implemented relational databases of 1024 human genes directly involved in signal transduction by Ras superfamily GTPases.

Reportable outcomes

A report of this study was presented as a talk by the PI at the 2003 National Neurofibromatosis Foundation NF consortium meeting in Aspen, CO. A previously published survey of GTPase Activating Proteins is included as Appendix 1.

Conclusions

We experienced an unexpected 8 month delay in receiving regulatory approval for this study. Patient recruitment was also impacted by the loss of one major collaborator, and by the fact that some promised patient samples were not eligible or otherwise not useable. We have taken

various measures to make up for the resulting shortfall in patient recruitment. We used the delay in patient recruitment to comprehensively identify modifier genes in three categories, and we have entered into new collaborations to improve our SNP genotyping and data analysis capacity once genotyping gets underway.

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Review

GAPs galore! A survey of putative Ras superfamily GTPase activating proteins in man and Drosophila

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Abstract

Typical members of the Ras superfamily of small monomeric GTP-binding proteins function as regulators of diverse processes by cycling between biologically active GTP- and inactive GDP-bound conformations. Proteins that control this cycling include guanine nucleotide exchange factors or GEFs, which activate Ras superfamily members by catalyzing GTP for GDP exchange, and GTPase activating proteins or GAPs, which accelerate the low intrinsic GTP hydrolysis rate of typical Ras superfamily members, thus causing their inactivation. Two among the latter class of proteins have been implicated in common genetic disorders associated with an increased cancer risk, neurofibromatosis-1, and tuberous sclerosis. To facilitate genetic analysis, I surveyed Drosophila and human sequence databases for genes predicting proteins related to GAPs for Ras superfamily members. Remarkably, close to 0.5% of genes in both species (173 human and 64 Drosophila genes) predict proteins related to GAPs for Arf, Rab, Ran, Rap, Ras, Rho, and Sar family GTPases. Information on these genes has been entered into a pair of relational databases, which can be used to identify evolutionary conserved proteins that are likely to serve basic biological functions, and which can be updated when definitive information on the coding potential of both genomes becomes available.

Keywords: GTPase activating protein; Ras superfamily; Drosophila and human genome survey

1. Introduction

Important clues about biological processes in mammals have often been obtained by studying related processes in less complex organisms that can be studied genetically [1]. In this and other types of research it is usually important to know how many related proteins might serve redundant functions. An accurate answer to this question should soon be available for most model organisms, when remaining genome sequence gaps are filled and when gene prediction programs are improved and their results experimentally verified [2,3]. Tentative answers, however, can already be provided and recent reviews have begun to catalog the number and the diversity of genes involved in such processes as gene expression [4], circadian clock function [5], DNA repair [6,7], or membrane compartmentalization [8].

Members of the Ras superfamily of small monomeric GTP-binding proteins play roles in diverse biological processes, ranging from transmembrane signal transduction, to various processes that involve cytoskeletal rearrangements, to vesicular trafficking, to nucleocytoplasmic transport, to list only the most prominent. Critical to the function of most Ras superfamily members is their ability to cycle between biologically active GTP-bound and inactive GDP-bound conformations. In their GTP-bound active state, members of the Ras superfamily can interact with a variety of effector proteins. By directly or indirectly altering the function of these effectors, Ras-like GTP binding proteins serve their various biological functions. The cycling of typical Ras superfamily members is controlled by guanine nucleotide exchange factors (GEFs), which activate the proteins by promoting GDP for GTP exchange, and by GTPase activating proteins (GAPs), which cause their inactivation by enhancing their low intrinsic GTPase activity. Other types of regulators act on specific groups within the Ras superfamily, for example guanine nucleotide dissociation inhibitors or GDIs, which maintain Rab, Ran, and Rho GTPases in a cytosolic GDP-bound form [9–12].

Abbreviations: CD, conserved domain; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GDP (GTP), guanosine di(tri)phosphate; PI 3-kinase, phosphoinositide 3-kinase

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Ras superfamily members, their effectors, regulators, or proteins involved in their processing have been implicated in various genetic disorders. Thus, mutations in two proteins involved in prenylation of Rab proteins underlie human choroideremia and a murine Hermansky–Pudlak syndrome model [13]. Among effectors, a protein that plays a central role in Cdc42-controlled F-actin changes is defective in Wiskott–Aldrich syndrome [14], and both nonsense and missense mutations in Rho effector kinase PAK3 occur in nonspecific X-linked mental retardation [15,16]. Among the disease implicated GTPases, H-Ras, K-Ras, and N-Ras harbor mutations that affect their GDP/GTP cycling in a substantial fraction of human cancers. A dominant negative mutation in the hematopoietic cell-specific Rac2 GTPase has been associated with impaired phagocyte function [17], and Rab27A mutations underlie the pigmentation and other defects of human Griscelli syndrome [18]. Multiple proteins that control the activity of Ras superfamily members have also been implicated in disease. Thus, RhoGAP oligophrenin-1 [19], RhoGEF ArhGEF6 [20], and RabGDI1 [21] are defective in different subsets of patients with X-linked mental retardation. Faciogenital dysplasia is associated with mutations in a GEF for Cdc42 [22,23]. A RhoGAP domain containing inositol polyphosphate 5-phosphatase is mutated in oculocerebrorenal syndrome of Lowe [24]. In a pair of interacting proteins that are mutated in tuberous sclerosis, one has been implicated as a GAP for Rap1 or Rab5 [25,26], and what may be the most common human disease associated with an increased cancer risk, neurofibromatosis type 1 or NF1, is caused by mutations in a protein that functions as a GAP for conventional Ras as well as R-Ras GTPases [27].

We and others have been interested in identifying what underlies the diverse symptoms of human NF1 by means of genetic analysis of Drosophila *NF1* mutants [28–31]. To help interpret *NF1*-deficient phenotypes, we surveyed public Drosophila and human genome databases for RasGAP-related proteins. Since it remains uncertain how strictly GAPs observe the boundaries between branches of the Ras superfamily and to make this survey more generally useful, we expanded its scope to include proteins related to the catalytic segments of GAPs for all members of the Ras superfamily. Excluded from this survey were GAPs for GTP-binding proteins outside of the immediate Ras superfamily, such as RGS proteins which serve as GAPs for heterotrimeric G proteins [32].

2. A brief history of GAPs

Ras-related GTP-binding proteins came to prominence around 1982, when mutations that affected the GDP/GTP cycling of H-Ras, K-Ras, or N-Ras were identified in what is now usually estimated to be at least 15% of human cancers. A large amount of work since has implicated a large number of Ras-related proteins as critical regulators of a broad range of biological processes [12,33]. A recent review of the Ras superfamily listed 19 mammalian members of the Ras/Rap group, 14 Rho-related GTPases, 42 Rab family members, 16 members of the Arf group, and a single Ran GTPase, adding up to 92 mammalian Ras-related GTP binding proteins [12]. However, a survey of the human genome draft sequence identified 60 loci predicting Rab-related proteins [8], and a nonexhaustive search of the National Center for Biotechnology Information (NCBI) LocusLink database (http://www.ncbi.nlm.nih.gov/LocusLink) uncovered more than 130 human genes for Ras superfamily members (searches for this survey were performed between 8/2001 and 12/2001). A multiple sequence alignment of 131 hypothetical and proven Ras superfamily members confirmed the existence of four major clusters of related proteins, i.e. the Arf/Sar group with 25 members, the Rho group with 17 members, 47 Rab group members, and 24 Ras-related proteins. Among 18 proteins that fall outside of these groups, several group into smaller clusters, for example Rabl2A and Rabl2B [34], RagA–D and FLJ14117 [35,36], and kappaB-Ras1 and kappaB-Ras2 [37]. Current databases also list at least two >90% identical human Ran paralogs, and two more dissimilar (~ 61% identical) Ran-like proteins predicted by the Drosophila *Ran* and *CG7815* genes.

Most Ras superfamily members are believed to serve as molecular switches by cycling between active GTP and inactive GDP bound states [9]. However, Rho-related Rnd1, Rnd2 and RhoE/Rnd3, Ras-like AGS1/Dexras1, and kappaB-Ras1 and kappaB-Ras2, are among members of this group that lack amino acids required for GTPase activity [37–40]. AGS1/Dexras1 mRNA rapidly accumulates upon dexamethasone stimulation [41], suggesting it may be regulated at the transcriptional level. Other proteins in this group may be regulated by controlling their subcellular localization, or in other ways.

The first identified GAP for any Ras superfamily member was a widely expressed vertebrate protein that accelerated GTP hydrolysis by wild-type but not mutant conventional Ras (i.e. H-Ras, K-Ras, or N-Ras) proteins by several orders of magnitude [42]. Initially generically named GAP, but now usually called p120GAP or p120 RasGAP, it represented the prototype of a class of Ras regulators, other members of which were soon identified in *Saccharomyces cerevisiae* [43,44], *Schizosaccharomyces pombe* [45,46], and *Drosophila melanogaster* [47]. All RasGAPs in these organisms included a related catalytic domain of around 330 amino acids, but shared little or no similarity outside of this region. Rather than indicating that RasGAPs from different phylogenetic groups are mostly unrelated, it is now obvious that most eukaryotic species express several classes of RasGAPs. Thus, the NF1 tumor suppressor protein neurofibromin is related to the budding yeast Ira1p and Ira2p RasGAPs [48], and 60% identical to the protein predicted by a Drosophila *NF1* ortholog [28]. Four mammalian proteins related to Drosophila Gap1, and

single Drosophila proteins related to p120GAP, and four human SynGAPs have also been identified (see Section 15).

Although structural differences underlie the typical subdivision of the Ras superfamily into the Arf/Sar, Rab, Ran, Ras/Rap, and Rho groups, all Ras superfamily members share considerable structural similarity [9,10]. Thus, it was unexpected when a protein that stimulated the GTPase activity of Rap1, which is approximately 55% identical to K-Ras, was found to be unrelated to RasGAPs [49]. Moreover, the catalytic domain of a protein that accelerated Rho-GTP hydrolysis was also unrelated to either RasGAPs or RapGAP. Rather, the catalytic segment of p50RhoGAP resembled parts of *n*-chimerin and BCR, which both acted as GAPs for the Rho-related Rac1 GTPase [50]. The idea that GAPs for GTPases that make up the different branches of the Ras superfamily share little sequence similarity gained further strength by the identification of structurally distinct GAPs for human and yeast Ran [51,52], for yeast Ypt6, a member of the Ypt/Rab group [53], for human Arf1 [54], and for yeast Sar1 [55].

3. GAP domain structures and catalytic mechanisms: differences and similarities

While the catalytic domains of GAPs for members of the different branches of the Ras family share no obvious sequence similarity, the majority of GAPs for GTPases within each subgroup are related [11]. This raises the question whether structurally distinct GAPs use similar or different mechanisms to promote GTP hydrolysis. Answers to this question have emerged from structural studies.

The crystal structure of a 334-amino-acid fragment representing the p120GAP catalytic domain revealed a fully alpha-helical domain structure [56]. Residues that are conserved among RasGAPs are present in a central 218-residue domain that corresponds to the minimal segment of neurofibromin that retains full catalytic activity [57]. The structure of a 333-residue GAP domain fragment of neurofibromin has also been determined [58], revealing a similar overall structure and suggesting explanations for some of the different catalytic properties of p120GAP and neurofibromin [59,60]. The observation that Ras-GDP and the catalytic fragment of p120GAP form a transition-state-mimicking complex in the presence of aluminum fluoride [61] allowed the determination of the structure of the Ras-GDP:AlF_3-RasGAP-334 complex [62]. Among insights provided by this structure is that RasGAPs promote Ras-GTP hydrolysis by properly aligning the catalytically important Gln^{61} in the switch II region of Ras and by contributing a so-called arginine finger residue (R789), which protrudes into the active site of the GTPase and which directly participates in Ras-GTP hydrolysis by stabilizing emerging negative charges during the transition state [62].

The 3D structures of an approximately 230-amino-acid C-terminal segment representing the catalytic domain of p50RhoGAP (a.k.a. Cdc42GAP or RhoGAP1) in an aluminum fluoride containing transition state mimicking complex with either RhoA-GDP or Cdc42-GDP have also been determined [63,64]. The fully alpha-helical catalytic domain, which is sometimes referred to as a breakpoint cluster region-homology or BH domain to reflect the fact that not all (Rho)GAP domain proteins have catalytic activity (see below), includes a core consisting of four bundled helices that includes most residues conserved among RhoGAPs. Similar to what was found for RasGAPs, an essential arginine residue of p50RhoGAP protrudes into the GTPase active site and directly participates in catalysis. Although the alpha-helical RasGAP and RhoGAP domain structures had initially appeared distinct [63], core domain similarities have since suggested a common evolutionary origin [65–68]. RhoGAP domain structures of the p85α regulatory subunit of phosphoinositide 3-kinase (PI 3-kinase) and of the GRAF protein have also been determined [69,70].

Distantly related RasGAPs and RhoGAPs include homologous arginine fingers that directly participate in catalysis. By contrast, RGS4 stimulates GTP hydrolysis by heterotrimeric G protein subunit Giα mainly by stabilizing the transition state [71,72]. Potential arginine fingers have been identified in GAPs for most Ras superfamily members [66]. In the case of yeast Gyp1p, which accelerates GTP hydrolysis by members of the Ypt/Rab family, a catalytically important arginine occupies a comparable position to the arginine finger of RasGAPs and RhoGAP [73,74]. Beyond this similarity, the fully alpha-helical catalytic domain of Gyp1p does not resemble the RasGAP or RhoGAP domain structures [74]. The 200–240-residue Ypt/RabGAP catalytic domain is referred to as a TBC domain, after the human Tre-2 oncogene, and the yeast Bub2p and Cdc16 spindle checkpoint proteins, which harbor similar domains [75].

Only a single RanGAP has been identified in all eukaryotic species analyzed to date. RanGAPs are members of a family of proteins that harbor a characteristic array of leucine-rich repeats. Budding yeast rna1p includes 11 such repeats and adopts a crescent-like shape containing both alpha helices and beta sheets that is unlike any other GAP structure [76]. Although in this and other cases a definitive description of the catalytic mechanism awaits the determination of the GAP-GTPase cocrystal structure, an rna1p Arg^{74}Ala mutant had a 100-fold reduced kcat for Ran-GTP hydrolysis. The side chain of Arg^{74} points towards the rna1p protein surface, however, which is not what is expected of an arginine finger. Moreover, unlike what was found for RasGAP and RhoGAP arginine fingers, an rna1p Arg^{74}Lys mutant showed little reduction in GAP activity [76]. Mutation analysis of human RanGAP1 also argued against an arginine finger based catalytic mechanism. Thus, mutation of two conserved arginines did not affect catalytic activity, while mutation of the third arginine simultaneously affected Ran binding and catalysis [77].

Two studies suggested different ArfGAP catalytic mechanisms [78,79]. Thus, the minimal catalytic domain of rat

ArfGAP spans 130 N-terminal amino acids and includes a Cys-X_2-Cys-X_{16}-Cys-X_2-Cys zinc finger motif [54]. The structure of this fragment in complex with N-terminally truncated Arf1-GDP and Mg^{2+} was determined, since no transition state mimicking complex formed in the presence of aluminum fluoride. Unlike the fully alpha-helical catalytic domains of RasGAPs, RhoGAPs, RGS4, and Cyp1p, the ArfGAP catalytic domain includes both beta sheets and alpha helices. Moreover, whereas RasGAPs and RhoGAPs occlude the effector-binding regions of their substrate GTPases, Arf1GAP bound Arf1 away from its active site. The orientation of two arginines conserved among ArfGAPs suggested that they do not participate in catalysis [78]. The fact that ArfGAP binding did not obstruct the Arf1 effector domain argued that the Arf1–ArfGAP1 complex might interact simultaneously with an effector protein. Indeed, ArfGAP1 and coatomer complex, a major Arf effector (see Section 7), synergistically stimulated Arf1-GTP hydrolysis, and it was suggested that coatomer might stimulate Arf1-GTP hydrolysis by contributing an arginine finger residue in a tripartite complex [78].

A different ArfGAP catalytic mechanism resembling that employed by RasGAPs or RhoGAPs has been proposed, based on the structure of the ArfGAP domain and C-terminally flanking ankyrin repeats of PYK2-associated protein β [79]. The structure of the PAPβ (encoded by the *DDEF2* gene) and ArfGAP1 catalytic domains is similar in some regions, but different in others. In part this reflects the presence of an insertion in the catalytic domain of PAPβ and other ankyrin repeat-containing ArfGAPs. More surprising was that the ankyrin repeats formed an extensive interface with the ArfGAP domain, which overlapped substantially with the position occupied by Arf1 in the ArfGAP1 structure. Another difference was that the conserved arginine in the zinc finger domain of PAPβ was solvent exposed. Arguing for a catalytic role for this residue, changing it into a lysine reduced the PAPβ GAP activity by about 10,000-fold [79]. Following up on this finding, a recent study found that coatomer-stimulation of ArfGAP activity is only observed with an N-terminally truncated Arf1 mutant, or in the absence of phospholipid vesicles [80]. Thus, although coatomer may enhance ArfGAP activity under some conditions, it seems likely that ArfGAPs, RasGAPs, and RhoGAPs employ similar catalytic mechanisms in vivo.

No crystal structure has yet been reported for yeast Sec23p, which stimulates the GTPase activity of the Arf-like Sar1 GTPase [55]. Similarly, no RapGAP domain structure is yet available, although potential arginine fingers have been identified in several RapGAP-related proteins [66].

4. Substrate specificity of GAPs

It is typically assumed that GAPs are specific for GTPases within their own subgroup, i.e. that RhoGAPs only target GTPases within the Rho branch of the Ras superfamily, etc. However, an increasing number of GAPs are showing unexpected substrate specificities. Thus, RhoGAP-related CeGAP from *Caenorhabditis elegans* not only acted as a GAP for several Rho family members, but also stimulated the in vitro activity of *C. elegans* and human Ras, and of human Rab3A [81]. Similarly, mammalian RasGAP-related $GAP1^{IP4BP}$ stimulated the in vitro activity of both Ras and Rap, although only its RasGAP activity was enhanced by inositol 1,3,4,5-tetrakisphosphate [82]. Other RasGAPs such as p120GAP also interact with Rap1, but do not stimulate its activity [83]. Also worth noting in this respect is that *Pseudomonas aeruginosa* exoenzyme S is distantly related to RasGAPs, but acts as a GAP for Rho, Rac, and Cdc42 [84]. Obviously, the ability to activate a GTPase in vitro does not necessarily mean that the same will occur in vivo. Accumulating evidence suggests, however, that at least some GAPs have functionally important interactions with GTPases outside of their presumed target groups. Thus, the RapGAP-related *TSC2* gene product tuberin bound rabaptin-5 in a two-hybrid screen and stimulated Rab5-GTP hydrolysis in vitro [26]. Rab5 functions as a rate-limiting GTPase in endocytosis [85], and tuberin-deficient cells showed a low level of fluid-phase endocytosis, which was enhanced upon tuberin reexpression [26]. The catalytic domain of p120GAP also stimulates the activity of Rab5, but not of Rab3, Rab4, or Rab6 [86]. An intriguing recent paper suggests that the effects of wortmannin on EGF receptor endocytosis are independent of PI-3 kinase inhibition, but rather reflect activation of Rab5. Suggesting a role for p120GAP in Rab5 activation, wortmannin caused a reduction in the amount of Rab5 in anti-p120GAP immunoprecipitates [87].

RasGAP-related *S. cerevisiae* Bud2p regulates Rap-related Bud1p, providing another example of a GAP showing unexpected substrate specificity in vivo [88]. A recent study also suggests that RhoGAP-related DdRacGAP1 is required for RabD-dependent regulation of the contractile vacuole system in *Dictyostelium*. Arguing against an indirect effect, the RhoGAP domain of DdRacGAP1 stimulated GTP hydrolysis by RabD in vitro [89]. Relevant to the issue of substrate specificity is that minor sequence changes can have major effects on interactions between GAPs and GTPases. Thus, changing threonine-61 of Rap1A into a glutamine as is found in Ras allowed p120GAP to acts as a GAP for Rap1A [90], and amino acid substitutions in the arginine finger loop of yeast Ira2p converted it into an efficient GAP for mammalian H-Ras [91].

Human IQGAP1 and IQGAP2 interact with Cdc42 and Rac1 at least in part via their RasGAP-related domains [92,93]. The RasGAP domains of IQGAP1 and IQGAP2 lack obvious arginine fingers and neither protein exhibits obvious GAP activity. Rather, IQGAPs have the opposite effect and show dosage-dependent inhibition of both the intrinsic and the RhoGAP-stimulated GTPase activity of Cdc42 and Rac1 [92,93]. At least two more classes of RhoGAP-related proteins also lack obvious GAP activity.

Thus, the RhoGAP domain of the p85α regulatory subunit of PI 3-kinase has a structure resembling that of p50RhoGAP [69], but although the protein interacts with activated Cdc42 and Rac1 [94], it showed no GAP activity towards Rho family members [95]. Similarly, the RhoGAP domain of inositol polyphosphate 5-phosphatase (encoded by the *INPP5B* gene) showed no activity towards RhoA, Rac1, Cdc42, H-Ras, or Rab5b [96]. The RhoGAP domains of these proteins may have evolved into GTPase binding sites without catalytic activity. It is also possible that the true GTPase substrates for these proteins remain to be identified, or that GAP activity requires modifications not found on recombinant proteins. Lack of activity may also reflect that some GAPs require cofactors. Thus, RabGAP-related cdc16p from *S. pombe* functions in a complex with byr4p as a two-component GAP for the Spg1 GTPase [97]. Similarly, RabGAP-related Bub2p from *S. cerevisiae* functions as a subunit of a heterodimeric Cdc5-regulated GAP for Tem1 [98,99]. Both Spg1 and Tem1 are only distantly related to Ypt/Rab GTPases.

Several GAPs show specificity towards a subset of GTPases within a branch of the Ras superfamily. Thus, human ABR and BCR stimulate the GTPase activity of Rac1, Rac2, and Cdc42, but not that of RhoA [100]. Conversely, human RhoGAP6 (encoded by the *ARHGAP6* gene) is a GAP for RhoA, but not for Rac1 or Cdc42 [101], and GRAF promotes GTP hydrolysis by Cdc42 and RhoA, but only showed weak activity towards Rac1 [102]. Restricted substrate specificity may explain why so many GAPs exist. Another reason may be that different GAPs regulate the same GTPase within different signaling complexes. This latter point relates to the question whether GAPs have functions besides their roles as GTPase regulators. For the many GAPs that include a variety of protein interaction motifs, such additional functions might involve the assembly of specific effector complexes. GAPs that include additional enzymatic domains are most likely to have intrinsic effector roles. The finding that Rafl weakly stimulates Ras-GTP hydrolysis blurs the distinction between GAPs and effectors [103]. Proteins such as Rafl, however, have not been included in this survey.

5. Description of the survey

To assess the potential for functional redundancy and to identify evolutionary conserved proteins that are likely to serve basic biological functions, we set out to identify a comprehensive set of human and Drosophila proteins related to GAPs for members of the Ras superfamily. First, a list of confirmed and potential human GAPs was compiled by performing keyword searches of the NCBI PubMed and

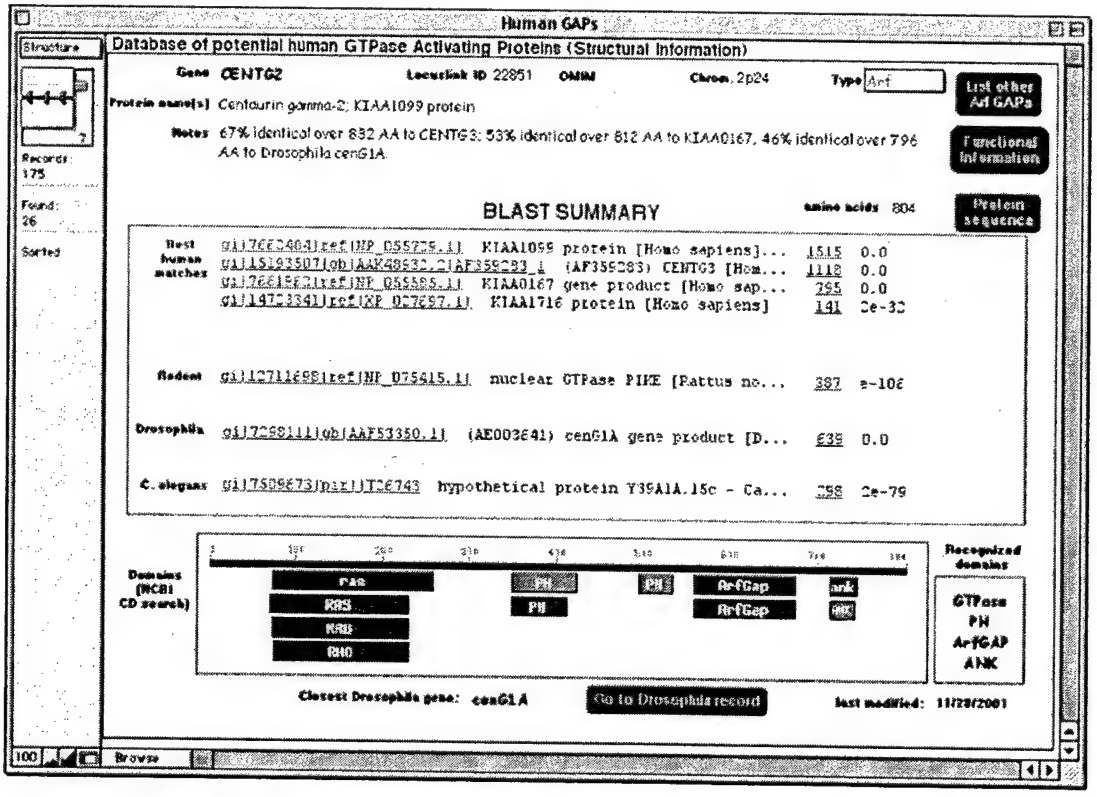

Fig. 1. Sample Human GAP database record for centaurin gamma-2, the product of the *CENTG2* gene. The BLAST summary section provides an edited list of the best human, rodent, Drosophila and *C. elegans* matches using the standard BLAST output format. A CD search (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml; Ref. [363]) was performed to generate the schematic domain structure diagram. See text provided with databases for additional information.

LocusLink databases. To keep track of information, a Filemaker database consisting of individual records for each protein was created. Second, BLAST searches of the nonredundant (NR) GenBank database were performed for each protein using default settings, i.e. the blosum62 substitution matrix, default gap introduction (11) and extension (1) penalties, and filtered for low complexity regions [104]. The highest scoring human, rodent, Drosophila, and *C. elegans* matches were entered into each protein's database record, and additional records for newly identified proteins were created. Thirdly, a separate database of Drosophila GAP-related proteins was created. Entered into this database were the results of crosswise BLAST searches of Drosophila GAPs against the human subsection of GenBank, and of the most closely related human GAPs against the translated Drosophila genome database. Finally, we searched the Ensemble version 1.2.0 human confirmed and predicted peptide databases (http://www.ensembl.org), and the process was repeated until no additional genes/proteins were found.

The human and Drosophila GAP databases (combined size around 4 MB in Filemaker Pro 5.5 format) can be downloaded (http://www.mgh.harvard.edu/depts/cancercenter/bernards.html). Figs. 1 and 2 show sample records for the human CENTG2 (centaurin gamma-2) putative ArfGAP, and for cenG1A, its closest Drosophila ortholog. Fig. 3 shows another layout of the human database, in which human and Drosophila ArfGAPs are sorted according to structural similarity. A description of database features and functions is provided with the databases. Each human database record is linked to the most closely related Drosophila record, and vice versa, each Drosophila record is linked to up to three related human proteins. This feature helps to identify evolutionary conserved proteins, but requires that both databases are downloaded.

Some proteins are known by several synonyms and many potential genes identified by DNA sequencing have preliminary names that will eventually be replaced. Thus, to minimize confusion we identify genes by their current LocusLink or Ensemble database names. For each gene we also provide the unique LocusLink and Ensemble 1.2.0 identification numbers. The fact that the LocusLink database can be searched with a comprehensive list of synonyms should make it possible to identify genes and proteins even if their names evolve.

This survey was done between July and December of 2001 using current GenBank and Ensemble 1.2.0 databases. With an estimated 75% of the human genome sequence available in draft only, any human genome survey will obviously be incomplete. Thus, the Ensemble 1.2.0 release differs substantially from the previous release, and future releases will undoubtedly include further changes. Unsequenced regions also remain in the Drosophila genome, and

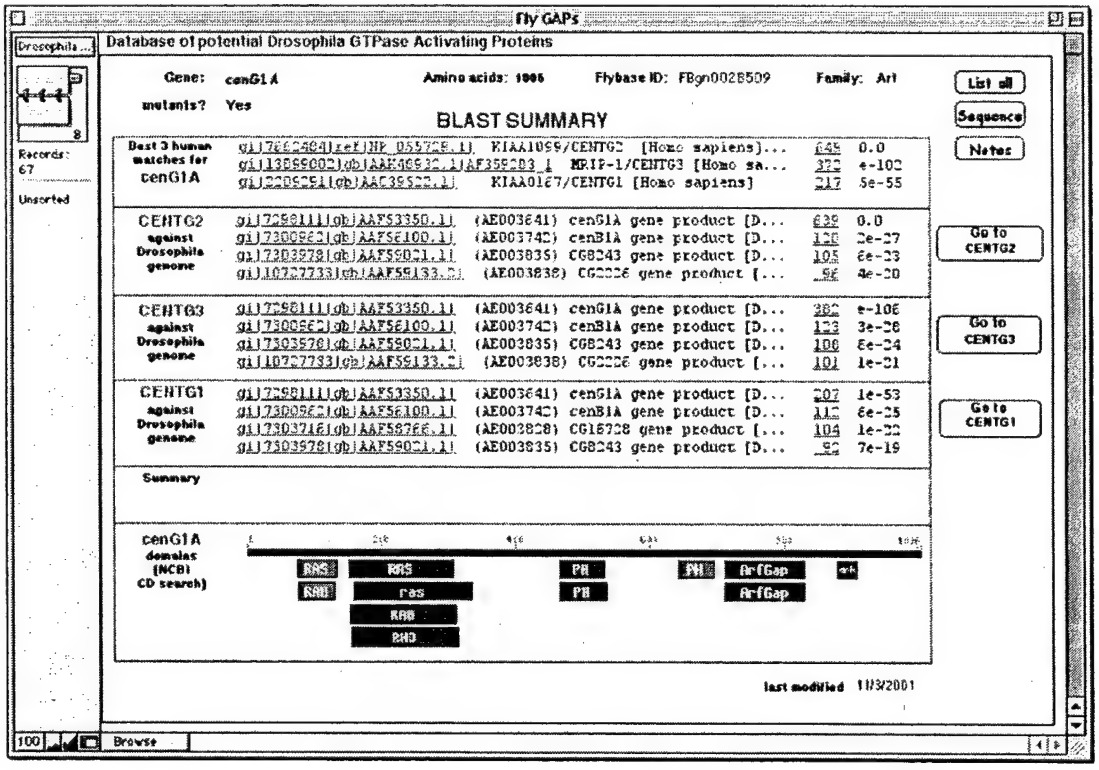

Fig. 2. Sample Drosophila GAP database record for cenG1A, the closest ortholog of human CENTG2. The BLAST summary section shows results of crosswise BLAST searches of the Drosophila protein against the human subset of GenBank (top), and of up to three related human proteins against the Drosophila genome database.

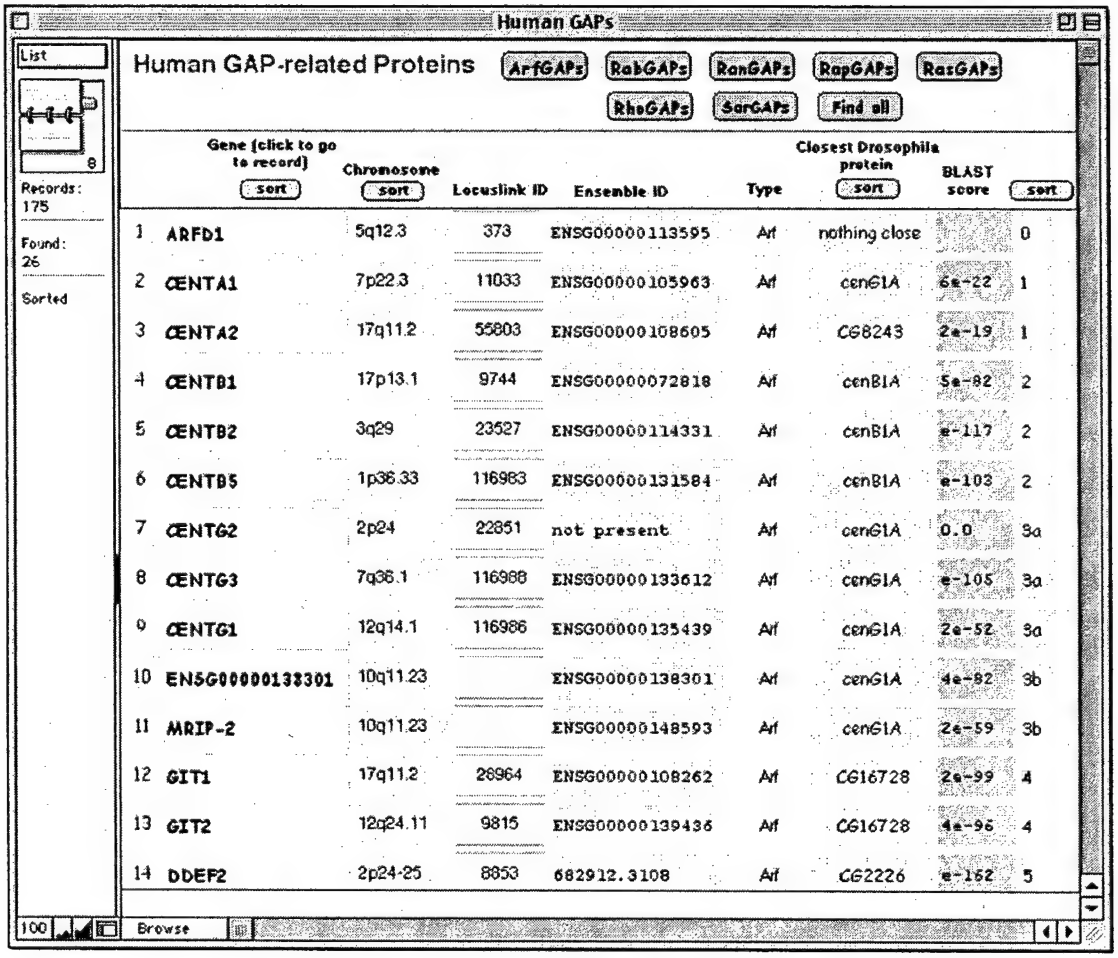

Fig. 3. Layout of the human GAP database listing human ArfGAPs and their closest Drosophila orthologs.

for both genomes annotation is far from perfect. For these reasons, and because we did not survey the Celera human genome database, the number of GAP-related genes in Table 1 is certain to change.

A substantial number of the genes in Table 1 have not been functionally analyzed. For example, only 2 of 51 human proteins that include TBC/RabGAP domains have so far been shown to have RabGAP activity (the 52nd protein in this category is Rab3GAP, which has RabGAP activity but is unrelated in sequence). Thus, although it remains uncertain what proportion of genes in Table 1 encode functional GAPs, it is remarkable that close to 0.5% of human and Drosophila genes predict proteins related to GAPs for Ras superfamily members.

The remainder of this review summarizes information about the genes and proteins listed in Table 1. To provide a functional context, each of the following sections provides a brief outline of the processes that involve the GTPases in question. More details are available in two recent comprehensive reviews of the Ras superfamily [12,33], or in references cited where appropriate.

Table 1
Number of human and Drosophila genes predicting GAP-related proteins

GAP type	Human	Drosophila
Sar	2	1
Arf	26	8
Rab	52	24
Ran	1	1
Rap	11	4
Ras (*)	14	5
Rho	70	21
Total	173	64

The total number of human GAPs is three less than the sum of individual types, since three human centaurin delta paralogs include both ArfGAP and RhoGAP domains. (*) Plexin family members include cytoplasmic RasGAP-related segments, but only a single human and Drosophila plexin is included in these numbers.

6. Sar1 and SarGAPs

Cycling of the Arf-related Sar1p GTPase is required for protein transport from the endoplasmic reticulum to the Golgi complex in *S. cerevisiae* [105–107]. Activation of

Sar1p involves exchange factor Sec12p [108], whereas Sec23p inactivates Sar1p by enhancing its GTPase activity [55]. Suggesting that the ability to accelerate Sar1p-GTP hydrolysis is an important function for Sec23p, two temperature-sensitive Sec23p mutants showed reduced GAP activity at the nonpermissive temperature [55]. Sec23p is unrelated to other GAPs, but shares significant similarity to Sec24p, with which it forms heterodimeric complexes [109]. Sar1p, a 400-kDa Sec23p–Sec24p complex, and a 700-kDa Sec13p–Sec31p complex make up the 10-nm-thick electron-dense coatomer protein II (COPII) coat of ER to Golgi transport vesicles [107,110].

Two 85% identical human Sec23a and Sec23b proteins have been identified [111], as have at least four human genes predicting Sec24-like proteins [112]. Suggesting that human and yeast Sec23 orthologs have similar functions, expression of human Sec23a rescued a yeast SEC23 mutant [111]. The Drosophila Sec23 protein is 74% identical to human Sec23a over its entire length. The Drosophila *CG1472* gene encodes a single Sec24-like protein.

7. Introduction to Arf GTPases

ADP-ribosylation factor or ARF was originally purified from bovine brain based on its ability to stimulate cholera toxin-mediated ADP-ribosylation of heterotrimeric G protein subunit Gsα [113]. Subsequently, Arf-related GTPases were found to play important roles in vesicular trafficking and organelle homeostasis in organisms ranging from yeast to mammals [114,115]. The Arf branch of the mammalian Ras superfamily includes the conventional Arf1-6 proteins, multiple Arf-like, or Arl GTPases, and an unusual Arf domain protein 1 (Ard1), which includes an Arf-related GTPase domain downstream of an autocatalytic GAP domain [116].

Among three Arf proteins in budding yeast, Arf1 and Arf2 play redundant essential roles, whereas Arf3 is non-essential [117]. Mammalian Arf1-6 have been divided into three classes based on similarities in function, protein sequence, and gene structure [118]. Thus, Golgi-localized class I Arf1, Arf2, and Arf3 control vesicular trafficking along the exocytic and endocytic pathways, whereas the nucleotide binding status-dependent cycling between endosomes and the plasma membrane is among evidence that has implicated class III Arf6 as a regulator of an endocytic pathway [119]. No specific functions have yet been assigned to Class II Arf4 and Arf5. Arl proteins are related in sequence, but do not substitute for Arfs in functional assays. Thus, members of the Arl group do not activate cholera toxin and fail to rescue the lethality of yeast ARF1, ARF2 double mutants. However, Arl1 blurs this distinction by sharing some effectors and biological properties with Arf1 [120,121].

Whereas the Arf-related Sar1 GTPase directs the assembly of COPII ER to Golgi transport vesicles (see previous section), Arf1 recruits coatomer protein I (COPI) coats to vesicles that mediate retrograde Golgi to ER transport [122], as well as bidirectional transport between Golgi stacks [123]. Among other functions, Arf1 controls the assembly of adaptor protein 1 (AP-1), AP-3, and AP-4 clathrin coats by interacting with a family of Golgi-associated, γ-adaptin homologous, ARF-interacting (GGA) proteins [124,125, and references therein].

The role of Arf1 in COPI coat formation has been well studied. Activation of mostly cytosolic Arf1-GDP alters the conformation of its myristoylated N terminus, allowing Arf1-GTP to tightly associate with the Golgi membrane [126]. Membrane-bound Arf1-GTP then recruits coat complexes whose further assembly changes the shape of the membrane into that of a budding vesicle and causes the recruitment of receptor-bound cargo molecules. Arf-mediated activation of membrane associated phospholipase D-1 may control the rate of COPI vesicle formation [127]. Coat disassembly is required prior to fusion of vesicles with their target membranes, and occurs when a vesicle-bound Arf-GAP inactivates Arf [114].

Much recent work has focused on cross-talk between Arf and Rho GTPases. Among examples of such cross-talk, Arf1 affects extracellular matrix adhesion by redistributing paxillin to focal adhesions and by enhancing Rho-directed actin stress fiber formation [128], and Arf6 stimulates cortical actin rearrangements [129], is involved in cell spreading [130], and induces motility in epithelial cells [131]. It has been suggested that Arf6 may affect cortical actin at least in part by controlling the accumulation of activated Rac1 at the plasma membrane [132,133]. However, there seems to be more to the role of Arf6 than just controlling the localization of Rac1. Thus, Arf6 induced epithelial cell motility required Rac1 and phospholipase D activation, but blocking the latter did not interfere with Arf6-mediated Rac1 activation [131]. Arf6 has also been found to interact with the putative Rac1 effector Por1 in a GTP-dependent manner [129], and at least three different ankyrin domain-containing ArfGAPs associate with components of focal adhesion complexes [134,135]. Among other proteins that may play roles in cross-talk between Arf and Rho GTPases are three human centaurin delta paralogs that harbor both ArfGAP and RhoGAP domains.

8. ArfGAPs

ArfGEFs include an approximately 200-amino-acid domain related to yeast Sec7p [136]. A subclass of ArfGEFs is inhibited by brefeldin A, a fungal metabolite that reversibly blocks protein secretion by causing collapse of the Golgi [137,138]. It has been suggested that more ArfGEFs than ArfGAPs exist [139]. However, this survey found at least 26 human and eight Drosophila potential ArfGAPs (Table 2).

Among 26 potential human ArfGAPs in Table 2, only Ard1 lacks closely related human paralogs or Drosophila

Table 2
Twenty-six human and eight Drosophila genes predict ArfGAP-related proteins

Number	Human gene	GAP activity	LocusLink ID	Ensemble ID	Closest Drosophila gene	BLAST score	Class
1	ARFD1	Yes	373	ENSG00000113595	–	–	0
2	CENTA1	Yes*	11033	ENSG00000105963	CenG1A	6e – 22	1
3	CENTA2	Yes	55803	ENSG00000108605	CG8243	2e – 19	1
4	CENTB1	Yes	9744	ENSG00000072818	CenB1A	5e – 82	2
5	CENTB2	Yes	23527	ENSG00000114331	CenB1A	e – 117	2
6	CENTB5	ND	116983	ENSG00000131584	CenB1A	e – 103	2
7	CENTG1	ND	116986	ENSG00000135439	CenG1A	2e – 52	3A
8	CENTG2	ND	22851	–	CenG1A	0.0	3A
9	CENTG3	ND	116988	ENSG00000133612	CenG1A	e – 105	3A
10	–	ND	–	ENSG00000138301	CenG1A	6e – 80	3B
11	MRIP-2	ND	–	ENSG00000148593	CenG1A	2e – 59	3B
12	GIT1	Yes	28964	ENSG00000108262	CG16728	2e – 99	4
13	GIT2	Yes	9815	ENSG00000139436	CG16728	4e – 96	4
14	DDEF1	Yes	50807	ENSG00000020468	CG2226	e – 133	5
15	DDEF2	Yes	8853	682912.3108	CG2226	e – 162	5
16	FLJ20199	ND	55616	ENSG00000088280	CG2226	8e – 43	5
17	ARFGAP1	Yes	28286	ENSG00000100262	CG6838	8e – 60	6
18	ZFP289	Yes*	84364	ENSG00000030168	CG6838	2e – 61	6
19	FLJ10767	Yes*	55738	ENSG00000101199	GAP69C	3e – 61	6
20	CENTD1	ND	23278	ENSG00000047365	CG4937	7e – 34	7
21	CENTD2	ND	23290	ENSG00000110220	CG4937	6e – 33	7
22	FLJ21065	ND	64411	ENSG00000113548	CG4937	5e – 22	7
23	FLJ13159	ND	60682	ENSG00000112305	CG8243	1e – 41	8a
24	LOC64744	ND	64744	ENSG00000084070	CG8243	2e – 40	8a
25	HRB/RAB	ND	3267	–	drongo	3e – 35	8b
26	HRBL	ND	3268	ENSG00000106351	drongo	4e – 14	8b

Human proteins are sorted by sequence and domain structure similarities. Proteins without obvious paralogs, such as Ard1 (encoded by the *ARFD1* gene), were assigned structural class #0. Proteins predicted by the *CENTD1*, *CENTD2* and *FLJ21065* genes include both ArfGAP and RhoGAP domains. In BLAST searches, all three are most closely related to the RhoGAP-like protein predicted by the Drosophila *CG4937* gene. Ensemble ID numbers other than those starting with ENSG are entries found in the predicted peptide database. In the GAP Activity column, Yes* means that GAP activity was detected for likely murine (ZPF289) or rat (FLJ10767) orthologs, or that GAP activity was inferred from the ability to rescue a yeast *Gcs1* ArfGAP mutant (CENTA1). ND: not determined.

orthologs (orthologs are genes that are likely to serve the same function in different species, i.e. direct descendants by evolution, whereas paralogs are related genes within a species [140]). Thus, no other human or fly protein includes an Arf GTPase-like segment downstream of an autocatalytic GAP domain (Fig. 4). The Ard1 segment that is essential for GAP activity includes two arginine residues and a B-box-type zinc finger motif [141].

Centaurin alpha1 (CENTA1; the name refers to the chimeric nature of the protein) and CENTA2 include two pleckstrin homology (PH) domains downstream of an ArfGAP domain (Fig. 4). No Drosophila protein has a similar structure. Centaurin alpha1 was identified as an ArfGAP-related phosphatidylinositol 3,4,5-triphosphate (PIP3) binding protein that rescued a yeast Gcs1 ArfGAP mutant [142]. The related *CENTA2* gene is among 11 genes that are commonly deleted in neurofibromatosis-1 patients that exhibit a particularly severe phenotype [143].

At least 13 human proteins include an array of ankyrin repeats downstream of an ArfGAP domain. Based on structural similarity, these proteins fall into five groups (Table 2 and Figs. 4 and 5). The first group consists of centaurin beta-1 (CENTB1; ACAP1), CENTB2 (ACAP2), and CENTB5. All these proteins include a central ArfGAP domain flanked by an upstream PH domain and by three C-terminal ankyrin repeats (Fig. 4). The likely Drosophila ortholog to these proteins is encoded by the *cenB1A* gene (Table 2). CENTB1/ACAP1 and CENTB2/ACAP2 are GAPs for Arf6, localize to the cell periphery, and inhibit the formation of growth factor-induced membrane ruffles when overexpressed [144]. Five potential human ArfGAPs that have not yet been functionally analyzed have the Drosophila *cenG1A*-encoded protein as their closest relative (Table 2). Among this group, centaurin gamma-1 (CENTG1), CENTG2 and CENTG3 include Ras-like potential GTP-binding segments upstream of PH and ArfGAP/ankyrin domains (Figs. 1, 2 and 4). A protein called MRIP2 lacks the upstream GTP-binding domain, but is otherwise very similar. *MRIP2* is one of six or seven closely linked Ensemble 1.2.0 genes that predict >95% identical proteins. Since it remains unclear how many of these genes are functional, only one (*ENSG00000138301*) has been included in Table 2. The rat PIKE GTPase interacts with protein 4.1 N and enhances nuclear PI 3-kinase activity [145]. PIKE is virtually identical to residues 50 to 420 of CENTG1, but has an N-terminal extension and lacks the downstream ArfGAP domain. Whether PIKE represents an alternative *CENTG1* gene product remains to be determined.

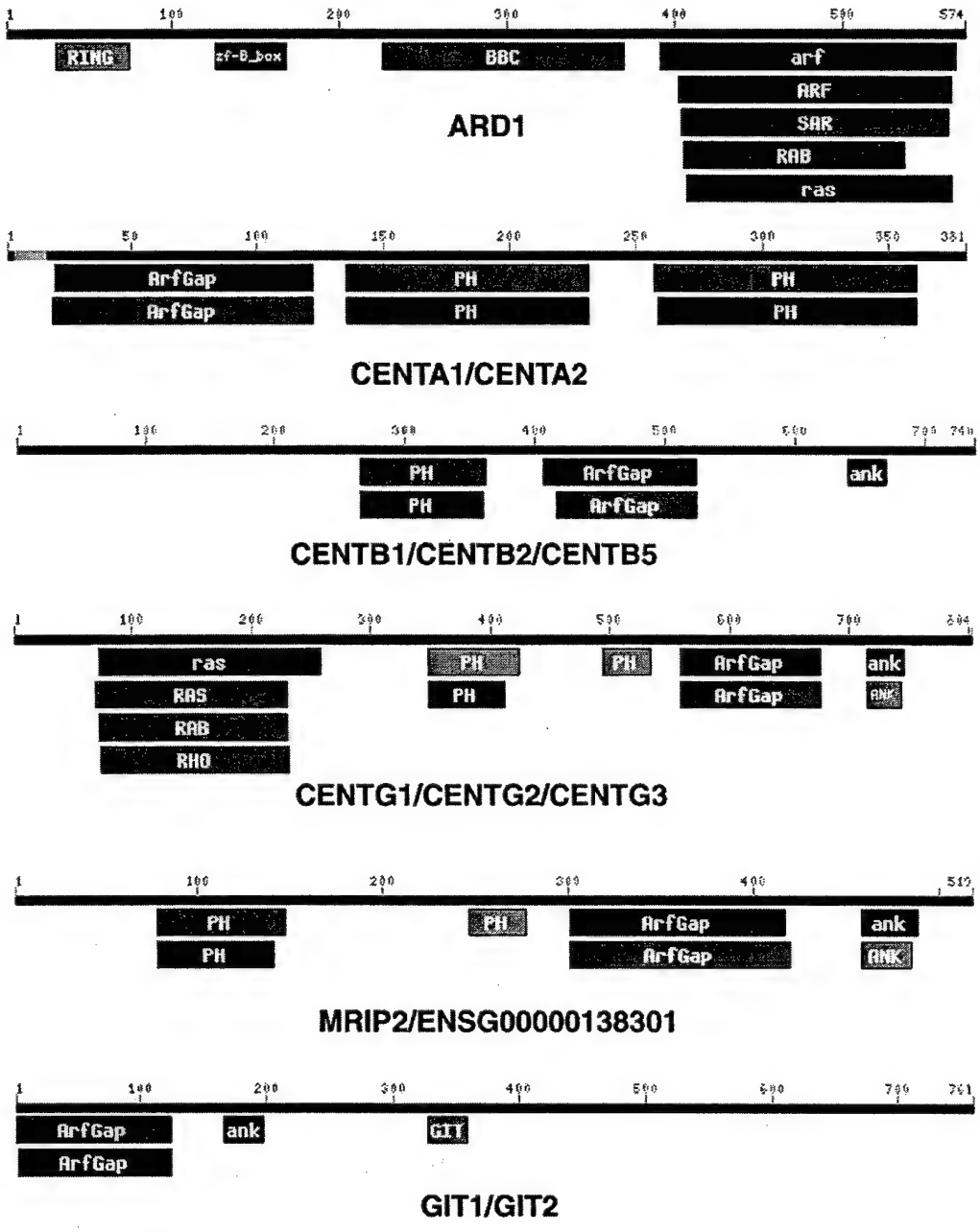

Fig. 4. Domain structure of putative human ArfGAPs. The diagrams were generated by performing a CD computer search, which scans proteins for segments that match domain profile databases [363]. Only domains that match domain profiles are detected. Only one representative structure for each group of related proteins is shown.

The fourth group of ankyrin-repeat containing ArfGAPs consists of G protein-coupled receptor kinase-interactor 1 (GIT1) and GIT2. GIT1 was found as a protein that interacted with G-protein-coupled receptor kinase-2 and that reduced β2-adrenergic receptor internalization when overexpressed [146]. More recently, GIT1 was found to specifically inhibit internalization of G-protein-coupled and other receptors that use the clathrin endocytic pathway [147]. Human GIT2 is 62% identical to GIT1 and is made in at least 10 alternatively spliced forms [148]. Both GIT1 and GIT2 possess PIP3-stimulated GAP activity towards Arf1, Arf2, Arf3, Arf5, and Arf6, but not towards Arl1, Arl2, or Ard1 [149]. The chicken GIT2 ortholog p95PKL (paxillin kinase linker) interacts with paxillin and with the Pix/Cool RhoGEF [150]. Similar findings have been reported for the human GIT2-short splice form, which localizes to the Golgi and primarily regulates Arf1 [151]. The protein predicted by the Drosophila *CG16728* gene is the likely ortholog of human GIT proteins.

The human differentiation and development enhancing factor-1 (*DDEF1*), *DDEF2*, and *FLJ20199* gene products

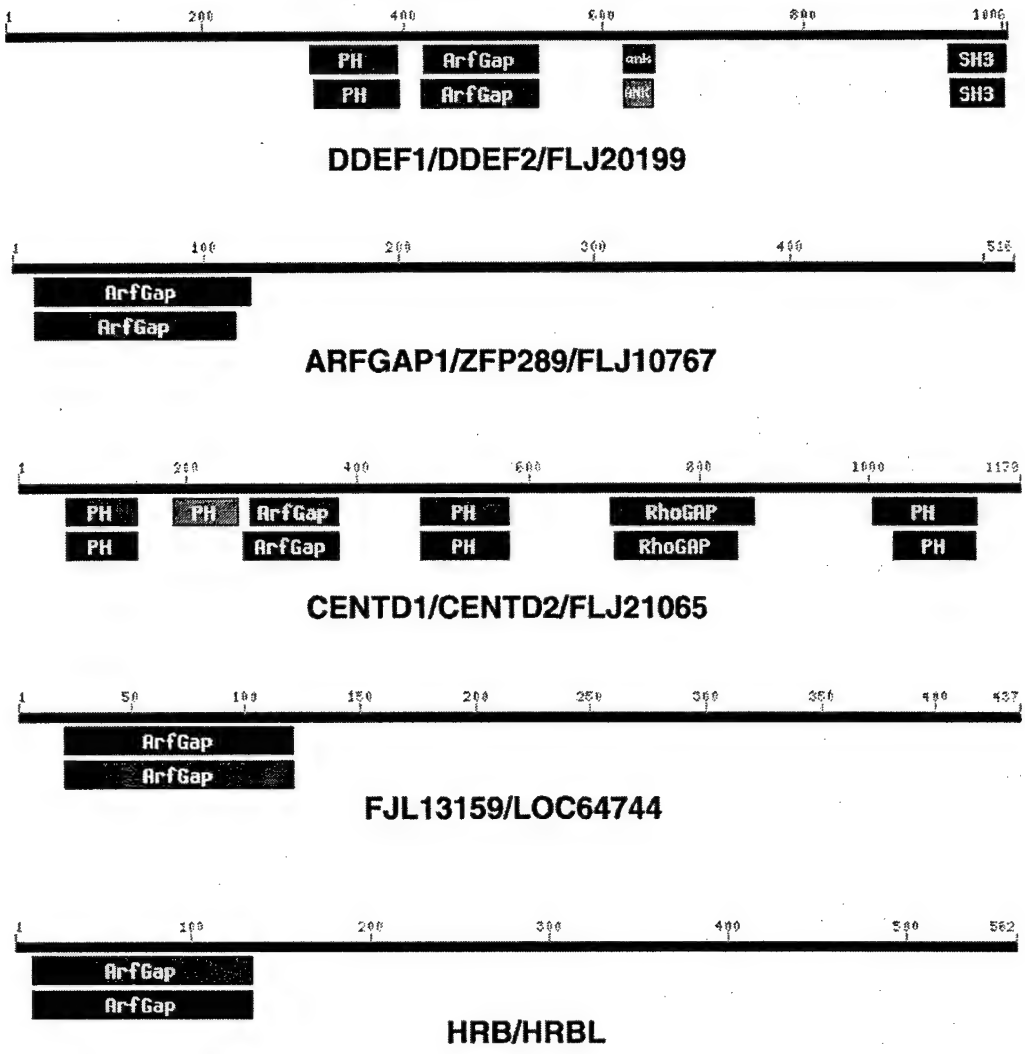

Fig. 5. Domain structure of putative human ArfGAPs. The diagrams were generated by performing a CD computer search, which scans proteins for segments that match domain profile databases [363]. Only domains that match domain profiles are detected. Only one representative structure for each group of related proteins is shown.

form the final group of ankyrin repeat harboring ArfGAPs (Fig. 5). The *DDEF1* protein, known as ASAP1, was identified as a two-hybrid interactor with the SH3 domain of Src, was tyrosine phosphorylated by Src, and exhibited phosphatidylinositol 4, 5-biphosphate (PIP2)-stimulated GAP activity towards Arf1 and Arf5, but less activity towards Arf6 [152]. The *DDEF2* protein (also known as Pap or PAG3) was found as a two-hybrid interactor with a C-terminal segment of the Pyk2 tyrosine kinase [153], and independently by screening a phage library with labeled paxillin [154]. The protein colocalizes with Arf6 at macrophage phagocytic cups, and phagocytosis was blocked by overexpression of wild-type but not of a GAP-deficient DDEF2/PAG3/PAP protein. This effect was overcome by also overexpressing Arf6, but not Arf1 or Arf5 [155]. Human DDEF1 and DDEF2 are likely orthologs of Drosophila CG2226. However, while both human proteins include a C-terminal SH3 domain (Fig. 5), the Drosophila protein does not. This likely reflects an incorrectly predicted *CG2226* open reading frame, because a peptide that is 60% identical to the DDEF1 SH3 domain is encoded immediately downstream of the current *CG2226* coding region. The GenBank version of FLJ20199 includes only a partial ArfGAP domain at its N terminus. However, a larger protein with a complete ArfGAP domain was found among the Ensemble predicted protein set.

The remaining nine human ArfGAP-related proteins form four groups (Fig. 5, Table 2). The first includes ArfGAP1, ZFP289 (the likely human ortholog of mouse zinc finger protein 289, which acts as a GAP for Arf1 [156]), and FLJ10767. ArfGAP1 and ZFP289 are closely related to each other and to Drosophila CG6838, whereas more distantly related human FLJ10767, which is the likely ortholog of rat ARFGAP [54], is more closely related to Drosophila Gap69C. Proteins that include a C-terminal KDEL-related motif are retained in the ER lumen by

interacting with a transmembrane KDEL receptor ERD2. The ERD2 receptor interacts with the noncatalytic domain of ARFGAP and recruits it to membranes, suggesting a role in protein trafficking [157,158]. However, a Drosophila Gap69C null mutant lacks obvious phenotypes, perhaps reflecting functional redundancy with other Drosophila ArfGAP-related proteins [159].

None of the three remaining classes of human ArfGAP-like proteins has close Drosophila orthologs. Thus, human centaurin delta-1 (CENTD1) and CENTD2 proteins include several PH domains in addition to ArfGAP and RhoGAP domains (Fig. 5). Neither protein has yet been analyzed, but a role in coordinating Arf and Rho-mediated processes seems likely. The human *FLJ21065* gene predicts a protein segment that is closely related to CENTD1 and CENTD2. An 1857-residue version of this protein in the Ensemble predicted protein database shares similarity with CENTD1 and CENTD2 over most of their length, suggesting it represents a third human centaurin delta paralog. Finally, two pairs of related human genes (*FLJ13159*, *LOC64744*, *HRB*, and *HRBL*) predict medium-sized proteins with N-terminal ArfGAP domains. The proteins predicted by the Drosophila *CG8243* and *drongo* genes are their closest relatives (Table 2). Among these proteins, HRB was identified as a cellular cofactor that enhanced Rev-mediated nucleocytoplasmic transport of unspliced human immunodeficiency virus mRNAs [160,161]. The presence of an ArfGAP domain-like N-terminal zinc finger domain in nucleoporin-related HRB and HRBL has been noted [162], as has their interaction with Eps15 and other EH domain containing proteins [162,163]. Interestingly, the only phenotype of $Hrb^{-/-}$ mice is a failure to form a sperm acrosome [164]. HRB localizes to preacrosomal vesicles and has been suggested to play a role in their fusion, but whether HRB serves as a GAP for Arf or other GTPases has so far not been addressed.

9. Introduction to Rab GTPases

Eleven genes encode Ypt-like GTPases in *S. cerevisiae* [165], and at least 60 human genes predict Rab-related proteins [8,166], making the Ypt/Rab group the largest branch of the Ras superfamily. Many members of the Ypt/Rab group associate with specific subcellular membranes and are believed to control discrete steps during vesicular transport along the secretory and endocytic pathways. By contrast to Arf and Sar GTPases which are best known for their roles during vesicle budding, Ypt/Rab proteins serve important roles mainly during the later stages of vesicular transport, probably by tethering vesicles to their target membranes prior to SNARE-dependent vesicle docking and membrane fusion [114,167–169]. However, interactions between Rab GTPases and specific kinesin and myosin motor proteins suggest additional roles in vesicle trafficking [170–172]. Other functions, including roles in budding, have also been suggested [173].

Mammalian Rab3A is a protein of presynaptic nerve terminals that regulates Ca^{2+}-dependent neurotransmitter release [174]. The cycling of Rab3 isoforms is controlled by Rab3GAP [175], by RabGDI, which maintains several inactive Rab family members in the cytosol by masking C-terminal hydrophobic geranylgeranyl groups [176,177], and by a Rab3-specific exchange factor [178]. Genetic evidence that GTP exchange controls the reversible association of Rab3 with synaptic vesicles comes from studies on the *C. elegans aex-3* neurological mutant. The AEX-3 protein resembles mammalian Rab3A-GEF and in its absence nematode Rab3 accumulates abnormally in the neuronal cell body [179].

Hypotheses regarding the role of Ypt/Rab GDP/GTP cycling have evolved over time. Thus, it was initially thought that nucleotide exchange recruited the GTPases to donor membranes, whereas GAP-stimulated GTP hydrolysis was important prior to vesicle fusion at the acceptor compartment [180]. The second part of this model required modification when membrane-bound Rab5 was found to undergo cycles of GTP-hydrolysis in the absence of membrane fusion [181]. However, the idea that Rab-GTP hydrolysis serves as a timer for membrane fusion [181] may also not be tenable in light of the recent finding that GTP hydrolysis is not important to the function of budding yeast Ypt1p. Thus, although Ypt1p plays an essential role in the exocytic pathway, a yeast strain in which the *ypt1* gene was replaced by a GTPase-deficient *ypt1-Q61L* mutant gene showed normal growth, secretion, and membrane morphology [182]. GTP hydrolysis by other Ypt proteins may be dispensable also, since loss of several Ypt GAPs causes few obvious defects [183,184]. Thus, for at least some Ypt family members, GAP-stimulated GTP hydrolysis may play a nonessential role in recycling the GTPases between membranes [182].

10. RabGAPs

Yeast Gyp1p shows GAP activity towards several Ypt GTPases in vitro [183,185]. However, Gyp1p localizes to the cis-Golgi and primarily regulates Ypt1p in vivo [186]. If Ypt/RabGAPs only regulate those GTPases that share their subcellular location, this may explain why the human and Drosophila genomes predict 52 and 24 potential RabGAPs, respectively (Table 3). Only human Rab3GAP, GAPCenA, and RN-tre among these proteins have so far been shown to have RabGAP activity [175,187,188]. However, among 10 RabGAP-related proteins in *S. cerevisiae,* six are confirmed YptGAPs, whereas Bub2p acts a subunit of a heterodimeric GAP for Tem1p, a distant Ypt relative [98]. The remaining three proteins have not yet been analyzed [186]. Extrapolating from these results it seems reasonable to assume that most proteins in Table 3 will also be functional GAPs.

Unique among the human proteins in Table 3, Rab3GAP does not include a TBC/RabGAP domain [175]. Similar to

Table 3
52 human RabGAP-related proteins and their closest Drosophila orthologs

Number	Human gene	GAP activity	LocusLink ID	Ensemble ID	Closest Drosophila gene	BLAST score	Class
1	RabGAP3	Yes	22930	ENSG00000115839	CG10870	1e – 39	0
2	dJ1042K10.2	ND	27352	ENSG00000100359	CG12241	0.0	0
3	FLJ10743	ND	54662	ENSG00000107021	CG5978	e – 108	0
4	FLJ11046	ND	55773	ENSG00000036054	CG4552	e – 147	0
5	FLJ11082	ND	55296	ENSG00000109680	CG7742	e – 100	0
6	BC001525.1	ND	–	ENSG00000141554	CG5337	8e – 77	0
7	MGC16169	ND	93627	ENSG00000138783	CG4041	e – 142	0
8	KIAA0210	ND	9779	ENSG00000131374	CG8449	7e – 69	0
9	FLJ22474	ND	79774	ENSG00000139835	CG5916	7e – 63	0
10	DKFZp4340047*	ND	26083	ENSG00000079548	CG5745	1.0	0
11	dJ852M4.2	ND	–	ENSG00000125875	CG17883	3e – 60	0
12	LOC51256*	ND	51256	ENSG00000145979	CG6182	6e – 21	0
13	KIAA1171	ND	57465	–	CG9339	2e – 51	0
14	–	ND	–	670666.360	CG11490	4e – 09	0
15	FLJ12085	ND	64786	ENSG00000121749	CG11490	6e – 85	1A
16	FLJ12168	ND	79735	ENSG00000104946	CG11490	2e – 79	1A
17	OATL1	ND	4943	ENSG00000068354	CG8155	4e – 74	1B
18	KIAA0397	ND	9905	ENSG00000141258	CG1702	e – 140	2
19	dJ930L11.1	ND	–	317643.16054	CG1702	e – 113	2
20	FLJ20337	ND	55633	ENSG00000065491	CG5745	2e – 83	3
21	C22ORF4	ND	25771	ENSG00000054611	CG5745	1e – 97	3
22	VRP	ND	11138	ENSG00000144212	CG7324	e – 106	4
23	KIAA0676	ND	23061	ENSG00000064747	CG7324	e – 152	4
24	KIAA0882	ND	23158	ENSG00000109436	CG7324	e – 103	4
25	–	ND	–	ENSG00000147225	CG7324	2e – 80	4
26	KIAA0603	ND	9882	ENSG00000136111	plx	2e – 98	5
27	TBC1D1	ND	23216	ENSG00000065882	plx	e – 106	5
28	GAPCENA	Yes	22637	ENSG00000011454	CG7112	e – 165	6a
29a	LOC64749*	ND	64749	ENSG00000135810	CG7112	9e – 54	6a
29b	KIAA0471*	ND	9910	ENSG00000135811	CG7112	1e – 47	6a
30	EVI5	ND	7813	ENSG00000067208	CG11727	e – 119	6b
31	LOC115704	ND	–	ENSG00000142459	CG11727	e – 126	6b
32	DKFZp761D1823	ND	55357	ENSG00000095383	CG12241	2e – 32	7
33	KIAA1055	ND	23102	530542.5262	CG12241	5e – 46	7
34	KIAA0608	ND	23232	ENSG00000108239	CG11727	2e – 22	8
35	KIAA1322	ND	57533	ENSG00000132405	CG11727	1e – 21	8
36	EPI64	ND	83874	ENSG00000099992	CG5344	6e – 84	9
37	DKFZp434P1750	ND	26000	–	CG5344	2e – 81	9
38	–	ND	–	3829.2001	CG5344	3e – 73	9
39	RNTRE	Yes	9712	ENSG00000148429	RN-tre	3e – 88	10a
40	USP6	ND	9098	ENSG00000129204	RN-tre	7e – 47	10b
41	TRESMCR	ND	27169	–	RN-tre	6e – 45	10b
42	DKFZp434P2235	ND	84218	ENSG00000132134	RN-tre	5e – 43	10b
43	–	ND	–	ENSG00000136502	RN-tre	4e – 42	10b
44	LOC94817	ND	–	–	RN-tre	1e – 24	10b
45	–	ND	–	ENSG00000129264	RN-tre	2e – 42	10b
46	–*	ND	–	317981.19168	RN-tre	0.054	10b
47	–*	ND	–	3524.25825	RN-tre	5e – 23	10b
48	–*	ND	–	ENSG00000147937	RN-tre	1e – 06	10b
49	–*	ND	–	ENSG00000147938	RN-tre	5e – 05	10b
50	KIAA0984	ND	23329	ENSG00000111490	CG3996	2e – 73	11
51	–	ND	–	ENSG00000135660	CG3996	2e – 66	11
52	–	ND	–	ENSG00000135640	CG3996	3e – 54	11

Eight human genes, indicated with asterisks, predict proteins that include only partial TBC/RabGAP domains. See Table 2 legend and GAP databases for further details.

what has been proposed for TBC domain protein Gyp1p, however, Rab3GAP may also employ an arginine finger-based catalytic mechanism. Among evidence supporting this conclusion, mutation of Arg^{728} of Rab3GAP disrupted its GAP activity but not its interaction with Rab3 [189]. Interestingly, whereas Rab3GAP shares no significant similarity to other human proteins, two Drosophila genes (*G10870* and *CG7373*) predict proteins related to Rab3GAP. Moreover, a reverse BLAST identified human Rab3GAP as the best match for either Drosophila protein.

Among 51 TBC domain-containing human proteins, 35 lack other domains recognized in conserved domain (CD) search [363]. Eight proteins, identified by asterisks in Table 3, are currently predicted to include only partial TBC domains (see below). Proteins are grouped by sequence and domain structure similarity, with the first group again consisting of human proteins without closely related paralogs. This group includes the catalytic subunit of Rab3GAP, and eight "TBC domain only" proteins. DKFZp4340047 and LOC51256 lack obvious paralogs and are currently predicted to include only partial TBC domains. However, a BLASTX search of the DKFZp3430047 cDNA sequence reveals additional TBC domain similarity, suggesting this clone may be aberrantly spliced or represent a pseudogene. In a CD search the human LOC51256 TBC domain was only 51.8% aligned. However, a 90% identical mouse protein of the same size aligns with the TBC domain profile over 97% of its length. We also note that whereas human LOC51256 and Drosophila CG6182 proteins are each other's closest relative in cross-wise BLAST searches, the predicted *CG6182* open reading frame does not include a TBC domain. However, a Drosophila genome translated BLAST search found a LOC51256-related TBC domain immediately downstream of the predicted *CG6182* coding segment.

Three human proteins without obvious paralogs include at least two different structural domains. Thus, the dJ1042K10.2 protein includes an SH3 and a RUN domain in addition to a TBC/RabGAP domain (Fig. 6). No other human protein closely resembles dJ1042K10.2, but the Drosophila CG12241 protein has an identical domain struc-

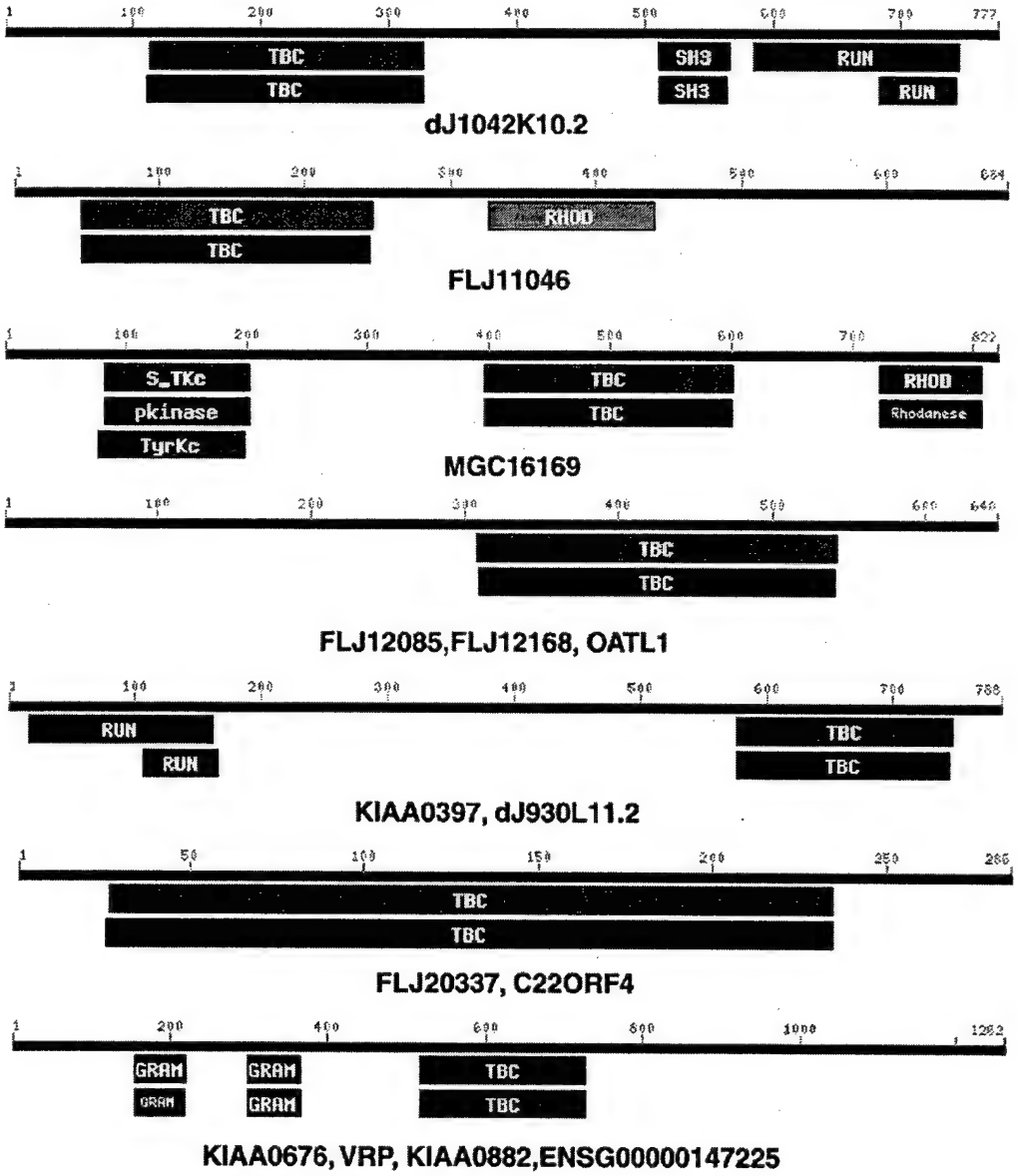

Fig. 6. Schematic structure of human TBC/RabGAP domain containing proteins. See Fig. 4 legend for details.

ture (Table 3). RUN domains are found in several proteins involved in Rap- or Rab-mediated processes, but their function remains poorly understood [190]. The FLJ11046 and MGC16169 proteins include rhodanese homology domains, which are found in rhodanese and other sulfur transferases, in several ubiquitin proteases, and in Cdc25 and other protein phosphatases [191]. Unique among human GAP-related proteins, the MGC16169 protein also includes similarity to an insulin receptor-like protein kinase domain (Fig. 6). Human FLJ11046 and MGC16169 are likely orthologs of the Drosophila CG4552 and CG4041 proteins, respectively.

Human FLJ12085 and FLJ12168 share ~47% amino acid sequence identity throughout and share a similar level of identity to the Drosophila CG11490 protein (Table 3). The human OATL1 protein is a more divergent member of this group and is more closely related to the Drosophila CG8155 protein. Human KIAA0397 and dJ930L11.2 proteins include N-terminal RUN domains and C-terminal TBC domains (Fig. 6). The Drosophila CG1695 and CG1702 proteins produce similar high BLAST scores with both human proteins. However, the predicted CG1695 protein does not include a TBC domain and while the CG1702 protein does include RUN and TBC domains, it is also currently predicted to harbor a C-terminal glutathione-S-transferase-like segment. A search of the translated Drosophila genome database did not detect a TBC domain near the CG1695 coding region, and CG1695 was not included among the 24 Drosophila RabGAP-related genes.

The human FLJ20322 and C22ORF4 predicted proteins share 78% overall sequence identity and are both closely related to Drosophila CG5745. The C22ORF4 protein is 98% identical to a *Macaca fascicularis* protein that extends approximately 170 amino acids further upstream. Human KIAA0676, ENSG00000147225 and VRP (vascular Rab-GAP/TBC domain containing protein), and the Drosophila *CG7324* gene product all include one or two N-terminal GRAM domains, which are found in several membrane-associated proteins [192]. Another related human protein, KIAA0882, lacks N-terminal GRAM domains. However, a BLAST search of the Ensemble predicted protein database with the GRAM domains of KIAA0672 identified a very similar protein encoded near the *KIAA0882* gene. Thus, the currently predicted KIAA0882 protein is likely incomplete.

Larval death of Drosophila *pollux* mutants has been attributed to a cell adhesion defect leading to abnormal tracheal development [193]. The 1379-amino-acid pollux protein includes a TBC domain and a phosphotyrosine-binding (PTB) domain, and is related to human TBCD1 and KIAA0603 proteins (Table 3). The KIAA0603 protein includes two PTB domains (Fig. 7), whereas no such domains are obvious in TBCD1. However, the Ensemble predicted protein database shows a KIAA0603-like PTB domain encoded near *TBCD1*, arguing that this gene is also incomplete.

GAPCenA is a 997-amino-acid protein that includes an N-terminal PTB domain, a central TBC domain, and an approximately 300-residue myosin-related C-terminal segment (Fig. 7). GAPCenA is a GAP for Rab6 and associates with centrosomes [187]. GAPCenA is 36% identical over most of its length to the Drosophila CG7112 protein (Table 3). Two human genes located at chromosome 1q25.1 predict proteins related to nonoverlapping segments of GAPCenA. Thus, the *LOC64749* gene predicts a 582-amino-acid protein with an N-terminal PTB domain and an approximately 50-amino-acid C-terminal segment related to the N terminus of a TBC domain. The KIAA0477 protein starts within the second half of a TBC domain and is 68% identical to the C-terminal segment of GAPCenA. In Table 3, LOC64749 and KIAA0477 are counted as a single gene, although this remains to be proven.

The products of the *EVI5* and *LOC115704* genes include TBC and myosin-like domains, but lack N-terminal PTB domains (Fig. 7). Both proteins are closely related to the Drosophila CG11727 protein (Table 3). The murine *Evi5* gene is frequently disrupted by proviral integrations in AKXD mouse T cell lymphomas [194], and a chromosome translocation in a neuroblastoma patient truncated human *EVI5* [195]. The similar *LOC115704* gene has not been implicated in cancer.

Human DKFZp761D1823 and KIAA1055 proteins include N-terminal PH and C-terminal TBC domains. Like the previous two proteins, human KIAA0608 and KIAA1322 lack obvious Drosophila orthologs. The EPI64, DKFZp434P1750, and AC005849 proteins share similarity over approximately 60% of their length, and are likely orthologs of the Drosophila CG5344 protein.

Genomic DNA segments derived from chromosomes 5q, 17q, and 18q recombined to give rise to the human transfection recombinant (TRE) oncogene [196,197]. The chromosome 17q-derived gene has transforming activity and encodes a widely expressed 786-amino-acid protein [198], which shares structural and functional similarity with a yeast deubiquitinating enzyme [199]. A large family of deubiquitinating enzymes exist [200], but unique among this class of proteins, the TRE17/tre-2/ubiquitin protease 6 protein includes an N-terminal TBC domain (Fig. 7). Several genes predict proteins that are highly related to N-terminal TRE17/tre-2/ubiquitin protease 6 segments [201,202]. However, it remains unknown whether any of these proteins are functional ubiquitin proteases. The Ensemble-confirmed and predicted peptide sets currently include over 10 proteins that are related to N-terminal Tre segments. Several genes for these proteins are closely linked on chromosome 17. Given the difficulty of distinguishing between multiple copies of very similar genes, the number of genes in this class should be considered preliminary.

The single Drosophila protein in this category, RN-tre, is most closely related to the human related to N terminus of TRE (RNTRE) protein. The Drosophila gene is widely expressed [203], but no mutants exist and it has not been functionally analyzed. Human RNTRE binds with high affinity the SH3 domain of EGF receptor substrate Eps8

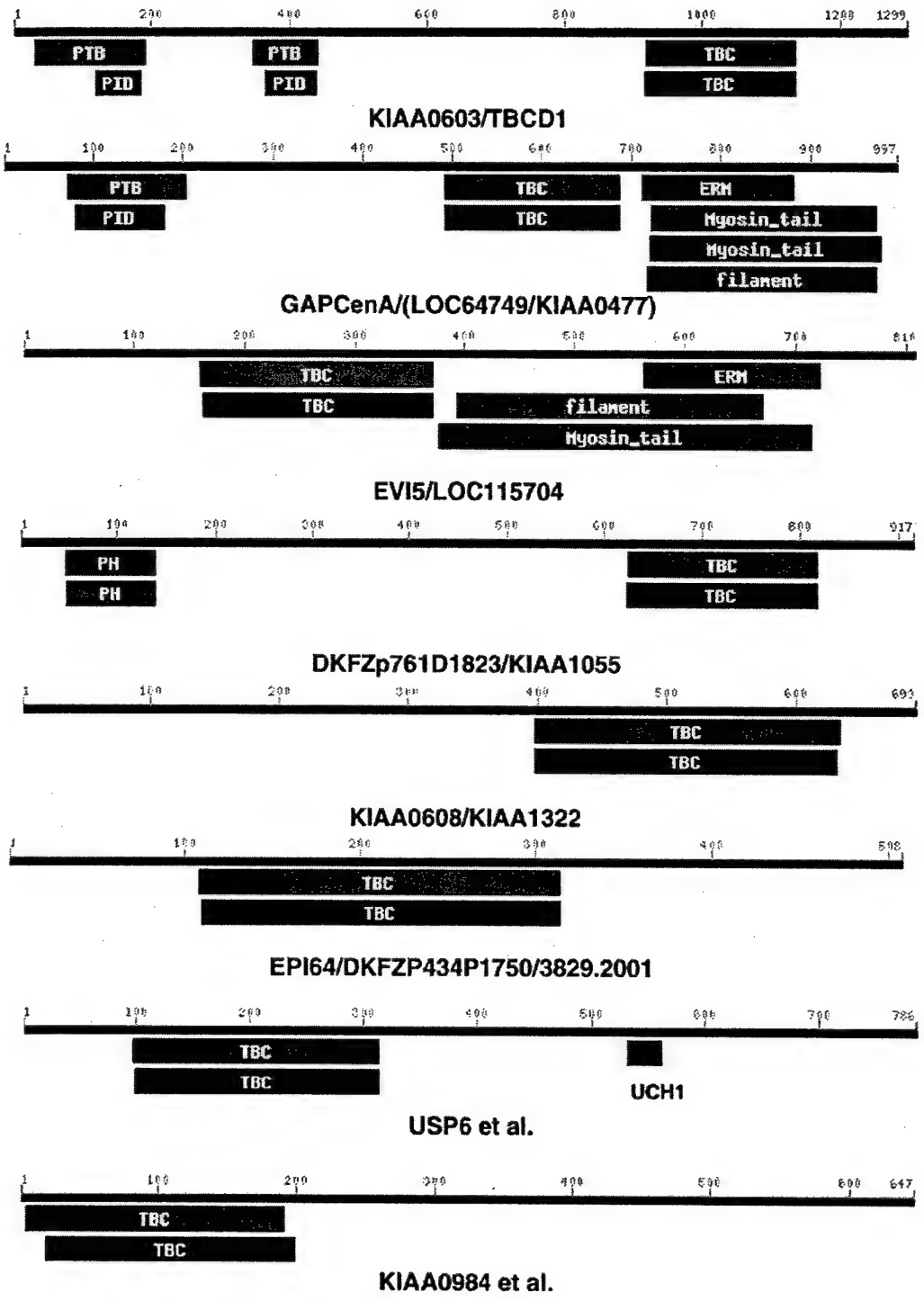

Fig. 7. Schematic structure of human TBC/RabGAP domain containing proteins. See Fig. 4 legend for details.

and has GAP activity for Rab5 [188]. Rab5 serves as a rate-limiting GTPase in endocytosis [85], and a role for RNTRE as a regulator of EGF receptor endocytosis has been proposed [188].

KIAA0984 is the prototype of the final class of potential RabGAPs. In addition to KIAA0984 itself, the latest Ensemble database lists two genes predicting proteins that are virtually identical to the N-terminal TBC domain of KIAA0984 (Fig. 7). All three KIAA0984-related genes are closely linked on chromosome 12q14.2. The Drosophila CG3922 predicted protein is the closest relative of KIAA0984. However, while KIAA0984 is only 728-

amino-acid long, the 3111-residue predicted *CG3922* gene product includes a large C-terminal segment. The Ensemble-predicted protein set includes proteins mapping to 12q14.2 that share additional similarity to the N terminus of CG3922, suggesting that one or more of the KIAA0984-related proteins are N-terminally truncated.

11. Ran and RanGAP

Ran/TC4 controls the directionality of nucleocytoplasmic protein and RNA transport and regulates mitotic spindle and nuclear envelope assembly in *Xenopus* egg extracts [204]. Only a single RanGEF, RCC1 for regulator of chromatin condensation, and only a single RanGAP, termed RanGAP1, have been found in mammalian cells [51,205]. The asymmetric distribution of RCC1 to the nucleus and of RanGAP1 to the cytoplasm is believed to result in a functionally important gradient of Ran-GTP across the nuclear envelope [206]. Other proteins that regulate Ran include RanBP1 and RanBP2/NUP380, which both enhance the activity of RanGAP1 [207,208], Mog1, which promotes GTP release [209], and nuclear transport factor p10/NTF2, which has RanGDI activity [210]. Covalent modification with the ubiquitin-like SUMO-1 protein plays a role in targeting RanGAP1 to the nuclear pore complex [208,211]. Genetic evidence also implicates GTP binding proteins Gtr1 and Gtr2 as regulators of budding yeast Ran [212]. Mammalian RagA–RagD are most closely related to yeast Gtr1 and Gtr2 [36], and RagA rescued the cold sensitivity of a yeast Gtr1 mutant [213].

Human RanGAP1 and other eukaryotic RanGAPs are members of a family of proteins that harbor leucine-rich repeats. Human RanGAP1 shares about 40% sequence identity over 377 N-terminal amino acids to Drosophila RanGAP, which is the product of the *segregation distorter* (*Sd*) locus. The *Sd* dominant gain-of-function allele encodes a truncated RanGAP, which by inducing dysfunction of Sd^+-spermatids causes the transmission of *Sd* mutant chromosomes to over 95% of the offspring of Sd/Sd^+ males [214].

12. Introduction to Rap GTPases

In a multiple sequence alignment human Rap1a, Rap1b, Rap2a, Rap2b, and a novel Rap2a-like protein predicted by the *LOC51655* gene form a cluster of related proteins within the larger Ras subgroup. This larger group includes the three conventional H-, K-, and N-Ras proteins, R-Ras1, R-Ras2/TC21 and M-Ras/R-Ras3, RalA and RalB, and several more distantly related proteins such as AGS1/Dexras1, Rhes, Rheb1 and -2, Rit, Rin, Rad, Ges, and Gem/Kir [215]. Although all proteins in this group are related, GAPs for Rap1 and Rap2 are structurally distinct from GAPs for conventional and R-Ras GTPases [216]. Two apparently distinct GAP activities for RalA have been detected, but not cloned [217,218]. Several known GAPs did not stimulate Rad-GTP hydrolysis, but a widely expressed cytosolic Rad GAP activity has been detected [219].

Overexpression of Krev-1/Rap1a causes flat reversion of K-Ras-transformed NIH3T3 fibroblasts [220]. This ability may reflect competition between Ras and Rap1a for a similar set of effectors [221]. Rheb, whose effector binding domain resembles that of Ras and Rap1, also antagonizes Ras when overexpressed [222], but the same is not true for Rap2 [221]. A Rap1GAP isoform that includes an N-terminal GoLoco/LGN motif binds heterotrimeric G protein subunit Giα as well as two other Gα subunits [223–225]. Membrane recruitment of Rap1GAP upon stimulation of the Gi-coupled m2-muscarinic receptor is accompanied by Rap1 inactivation and ERK/MAPK stimulation in the absence of an increase in Ras-GTP [223]. Thus, Giα-mediated ERK activation may reflect facilitated Ras signaling upon Rap1GAP-stimulated Rap1 inactivation [223]. However, Rap1 can also activate the MAP kinase cascade via B-Raf [226], and it remains controversial whether Ras antagonism is an important part of Rap1 function in vivo [227]. Recent progress towards identifying specific Rap-mediated functions has included the characterization of Rap exchange factors regulated by several second messengers. Progress has also been made towards defining the role of Rap1 as a regulator of integrin-based cell adhesion. The extensive literature on these and other Rap-related topics has been reviewed recently [228].

13. RapGAP-related proteins

The human and Drosophila genomes currently predict 11 and 4 RapGAP domain containing proteins, respectively (Table 4).

Two human proteins without closely related paralogs are DKFZp761J1523 and tuberin, the product of the tuberous sclerosis complex 2 (*TSC2*) gene. The 802-residue DKFZp761J1523 protein harbors a putative RapGAP catalytic segment followed by a citron homology (CNH) domain (Fig. 8). Similar protein segments are found in mammalian citron, NIK, and MRCKα protein kinases, in Drosophila *Genghis Khan*, and in yeast Rho1 exchange factors Rom1p and Rom2p. No currently predicted Drosophila RapGAP-related protein includes a CNH domain, and no DKFZp761J1523-like CNH domain is apparent in a Drosophila genome translated BLAST search.

Tuberous sclerosis is caused by defects in either one of a pair of interacting proteins, hamartin and tuberin [229]. A C-terminal segment of tuberin resembles the catalytic domain of Rap1GAP, and this part of the protein has in vitro GAP activity for Rap1a, but not for Rap2, H-Ras, Rac, or Rho [25]. However, the physiologically relevant substrate for tuberin may be endocytosis regulator Rab5. Thus, tuberin interacts with rabaptin-5 and stimulates Rab5-GTP hydrolysis. Moreover, the low rate of endocytosis of cells

Table 4
Human and Drosophila RapGAP-related proteins

Number	RapGAP	GAP activity	LocusLink ID	Ensemble ID	Closest fly gene	BLAST score	Class
1	DKFZp761J1523	ND	84253	ENSG00000136895	Rapgap1	7e – 50	0
2	TSC2	Yes	7249	ENSG00000103197	gigas	e – 123	0
3	RAP1GA1	Yes	5909	ENSG00000076864	Rapgap1	e – 122	1
4	KIAA1039	ND	21108	ENSG00000132359	Rapgap1	9e – 95	1
5	SIPA1	Yes	6494	ENSG00000074004	CG18646	2e – 50	2
6	KIAA0440	ND	26037	ENSG00000133986	Rapgap1	5e – 62	2
7	KIAA1389	ND	57568	ENSG00000116991	Rapgap1	9e – 64	2
8	KIAA0545	ND	23094	ENSG00000105738	Rapgap1	9e – 10	2
9	KIAA1272	ND	57186	ENSG00000088919	CG5521	e – 105	3
10	DKFZp566D133	ND	26134	ENSG00000119337	CG5521	e – 167	3
11	KIAA0884	ND	23000	ENSG00000136338	CG5521	e – 149	3

See Table 2 and the human and Drosophila GAP databases for additional information.

lacking tuberin was enhanced upon tuberin reexpression [26]. Important clues to the function of tuberin and hamartin have also come from studies of related genes in other species. Thus, suggesting a role for tuberin in protein trafficking, polycystin-1 was found to be sequestered in the Golgi of *Tsc2*-deficient rat kidney cells, and normal lateral membrane localization of polycystin-1 was restored upon *Tsc2* reexpression [230]. Mutations in Drosophila *Tsc1* or *Tsc2/gigas* orthologs cause a very similar increase in cell size, whereas combined overexpression of both genes reduced cell size [231–233]. The larger cell size, which is also seen in human tuberous sclerosis tumors, may reflect an antagonistic effect on insulin signaling [232,233]. It remains unclear whether the GAP function of tuberin orthologs is required for cell size regulation in Drosophila or for polycystin-1 trafficking in rat kidney cells. However, 11 human disease-associated *TSC2* missense mutations occur in the RapGAP-related segment [234,235, and references therein].

Human Rap1GAP was identified as an 88-kDa GAP for Rap1 but not Ras [49]. A close relative to Rap1GAP is KIAA1039, with which it shares 55% sequence identify over ~ 500 C-terminal amino acids. Both human proteins

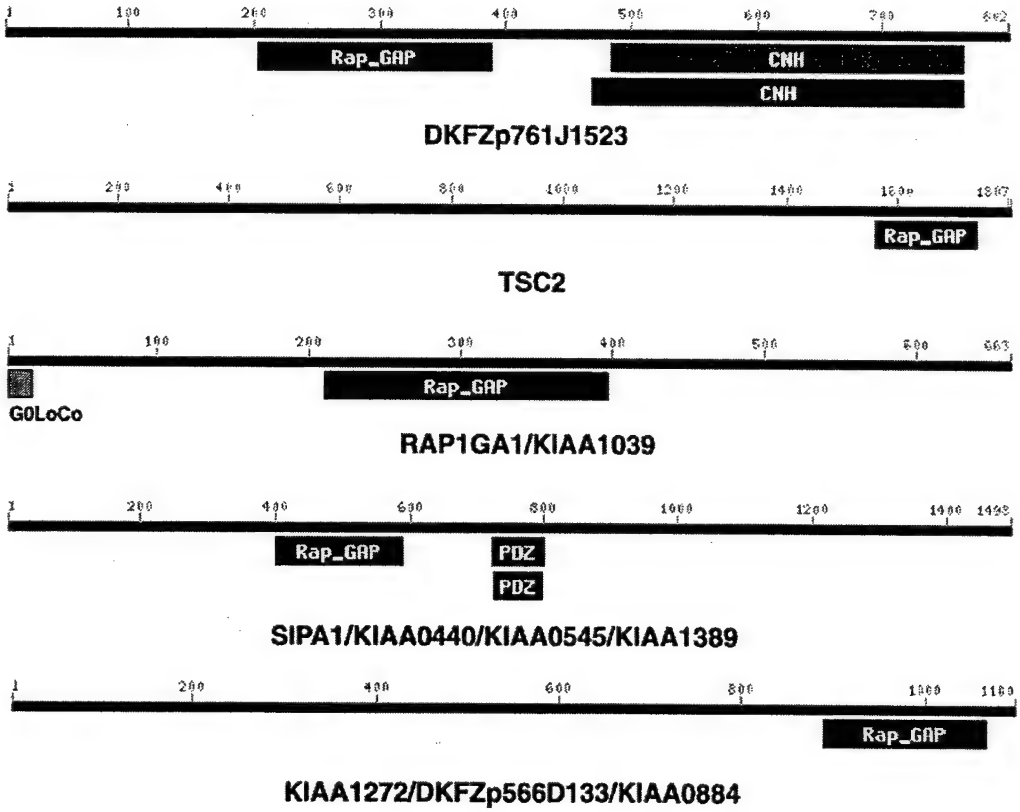

Fig. 8. Schematic structure of human RapGAP domain containing proteins. See Fig. 4 legend for details.

are closely related to Drosophila RapGAP1 [236]. Neither KIAA1039 nor Drosophila RapGAP1 is predicted to include a GoLoco/LGN Gα subunit binding domain (see Section 12). However, in a translated Drosophila genome BLAST search the best match to the Rap1GAP GoLoco/LGN domain is found ~ 6 kb upstream of the currently defined RapGAP1 coding sequence. Thus, a Drosophila RapGAP1 isoform may also be involved in Gα-mediated signal transduction, which makes it all the more surprising that Drosophila RapGAP1 null mutants lacked obvious defects [236].

Human SIPA1/SPA1, KIAA0440/E6TP1-alpha, KIAA0545, and KIAA1389 each include a single PDZ (PSD-95/Dlg/ZO-1) domain downstream of a RapGAP domain (Fig. 8). PDZ domains mediate protein interactions, often but not always by binding to the extreme C-terminal residues of membrane-associated proteins [237–239]. No currently predicted Drosophila RapGAP domain containing protein includes a PDZ domain. Rather, the human proteins in this group are distantly related to Drosophila RapGAP1 and CG18646. The latter protein, which has no close orthologs in other species, is currently predicted to include a putative RapGAP catalytic segment upstream of a partially aligned BTB/POZ domain.

Two human proteins in this class have been functionally analyzed. Thus, an N-terminally and C-terminally truncated signal-induced proliferation-associated gene 1 (SIPA1/SPA1) fragment that included the RapGAP domain localized to the nucleus and exhibited RanGAP activity [240]. However, subsequently it was found that full-length SIPA1/SPA1 localizes with Rap1 and Rap2 at perinuclear membranes, and is a GAP for Rap1 and Rap2, but not for Ran [241]. Supporting a role for Rap GTPases as regulators of integrin function, overexpressed human SIPA1/SPA1 negatively affected adhesion of HeLa cells to fibronectin, or growth factor-stimulated adhesion of 32D promyelocytic cells [242]. The other functionally analyzed protein in this group is KIAA0440/E6TP1-alpha, which was identified as a protein that was targeted for degradation by the E6 protein of transforming human papilloma virus variants [243]. Cellular proteins targeted for degradation by E6 include p53, but analysis of E6 mutants that differed in their ability to transform mammary epithelial cells found a perfect correlation between transforming capacity and the ability to degrade E6TP1-alpha [244]. Rat SPAR is 92% identical to human E6TP1-alpha and interacts with the guanylate kinase domain of PSD93, PSD95, and hDlg [245]. The C-terminal SPAR segment involved in this binding shares 44% identity with corresponding segments of KIAA1389 and KIAA0545, and 29% identity with SIPA1. SPAR has GAP activity for Rap2a and Rap1a, but not for H-Ras or several Rho GTPases. The latter were tested as potential substrates, because overexpressed SPAR induced a major reorganization of the F-actin cytoskeleton in COS-7 cells. In cultured hippocampal neurons, SPAR colocalized with PSD95 at dendritic spines and caused enlargement of spine heads. This effect required the RapGAP domain and one of two domains implicated in actin rearrangements [245]. Although much remains unclear about how SPAR regulates actin rearrangements and dendritic spine morphogenesis, it is interesting that in budding yeast a GAP for Rap-related Rsr1p controls bud site selection by recruiting Cdc42p exchange factor Cdc24p to the membrane [246].

The final group of RapGAP-related human proteins consists of DKFZp566D133, KIAA1272, and KIAA0884. The first two are >1100-residue proteins with C-terminal RapGAP domains. The status of the third protein is less clear. Thus, current GenBank versions of KIAA0884 are related to N-terminal parts of DKFZp566D133 and KIAA1272, but do not include a RapGAP domain. However, the *KIAA0884* gene maps to chromosome 14q13.3 and a 715-residue Genscan-predicted Ensemble protein mapping to the same chromosomal location includes a RapGAP domain. The situation, however, is not clear-cut since extensive segments of this latter protein are almost 100% identical to DKFZp566D133. Thus, further analysis is required before it can be concluded that KIAA0884 represents a third member of this group. The 1958-amino-acid *CG5521* gene product is the likely Drosophila ortholog of these proteins (Table 4). Likely rat orthologs called tulip-1 and tulip-2 (for tuberin-like protein 1 and 2) are listed in current databases, but no member of this group in any species has yet been functionally analyzed.

14. Introduction to Ras GTPases

Section 12 lists proteins that make up the Ras/Rap branch of the Ras superfamily. The best understood members of this group are the three related H-Ras, K-Ras, and N-Ras proteins that are mutated in various human cancers [247]. Oncogenic mutations either increase the rate of GDP/GTP exchange or reduce the rate of GTP hydrolysis, but for some Ras mutants the magnitude of this reduction did not appear commensurate with the high proportion of activated mutant Ras in vivo. This discrepancy led to the identification of mammalian p120 RasGAP, a widely expressed protein that stimulates the GTP hydrolysis rate of wild-type but not mutant Ras by several orders of magnitude [42]. At the time of its discovery, p120 RasGAP was the only protein known to interact with the effector domain of Ras [248], suggesting that it might combine regulator and effector functions [249]. The discovery of a Ras effector domain mutant that lacked transforming capacity but that retained the ability to interact with GAPs [250], and the discovery of several other Ras effectors (see Ref. [251] for a review), argued against a major effector role for p120GAP in mitogenic Ras signaling. However, p120 RasGAP recruits complexes that contain p190 RhoGAP and p62Dok to activated tyrosine kinases [252,253], suggesting it has roles that go beyond that of a negative regulator.

In PC12 rat pheochromocytoma cells, epidermal growth factor (EGF) stimulates proliferation whereas nerve growth

factor (NGF) induces differentiation. The finding that EGF induces differentiation of PC12 cells that overexpress the EGF receptor argues that the different response to EGF and NGF reflects a quantitative difference in signaling strength, rather than a qualitative difference in effector usage [254,255]. Obviously, the recruitment of different RasGAPs to receptor complexes provides one way to modify Ras signaling strength.

15. RasGAP-related proteins

Table 5 lists 14 human and five Drosophila RasGAP domain-containing proteins.

Human p120GAP and neurofibromin (the products of the *RASA1* and *NF1* genes, respectively) have obvious orthologs in Drosophila [28,258], but no closely related paralogs in man. Thus, although the Ensemble 1.2.0 release includes several genes that predict short NF1-related peptides, multiple *NF1* pseudogenes are known to exist [259]. The NF1 RasGAP domain (Fig. 9) is followed by a Sec14 putative lipid-binding motif [260]. Neurofibromin acts as a GAP for conventional and R-Ras GTPases [59,261], and several *NF1* deficient defects have been attributed to abnormal Ras signaling [27]. Interestingly, defects in three *NF1*-deficient mammalian cell types are rescued by expressing the NF1-GAP domain, but not by expressing the corresponding segment of p120GAP [262]. The Drosophila *NF1* ortholog encodes a 60% identical protein with RasGAP activity [28]. Unlike *Nf1*-deficient mice, Drosophila *NF1* null mutants are viable, but reduced in size. The mutants also exhibit neuropeptide signaling [29], learning [30], and circadian rest-activity rhythm defects [31]. The latter phenotype is rescued by reducing Ras signaling strength, whereas all other defects are insensitive to Ras manipulation, but rescued or mimicked by increasing or decreasing signaling through the cAMP/protein kinase A (PKA) pathway. Why loss of Drosophila *NF1* results in defects that are sensitive to cAMP manipulation remains unclear.

Human p120GAP and Drosophila RasGAP have the same domain structure and share 44% overall sequence identity (Fig. 9). Like neurofibromin, p120GAP is a GAP for conventional Ras and R-Ras proteins [216]. Human p120GAP also acts as a GAP for Rab5 [86], but whether the same is true for Drosophila RasGAP has not yet been determined. Missense mutations within the C-terminal SH2 domain of p120GAP have been reported to occur in human basal cell carcinoma [263], but the significance of this finding remains unclear. Loss of murine p120GAP results in embryonic lethality, abnormal vascularization, and enhanced neuronal apoptosis [264]. Drosophila RasGAP mutants have not yet been described, but ectopic expression of RasGAP in wing imaginal discs resulted in smaller wings with fewer cells [258]. A specific phosphotyrosine residue of the *Torso* tyrosine kinase provides a docking site for Drosophila RasGAP. This tyrosine is specifically dephosphorylated by *Corkscrew*, supporting a model in which RasGAP and Corkscrew have opposing roles in regulating Torso signal transduction [265].

Gap1 was identified as a negative regulator of *sevenless* signaling in Drosophila eye development [47]. Like Drosophila Gap1, the proteins predicted by four human Gap1-related genes (Table 5) include a pair of phospholipid binding C2 motifs followed by RasGAP and PH domains similar to the one found in Bruton's tyrosine kinase or BTK (Fig. 9). The four mammalian Gap1-like proteins differ in several potentially important ways. Thus, the C2 domains of $Gap1^{m}$ (encoded by the *RASA2* gene) and $GAP1^{IP4BP}$ lack

Table 5
Human and Drosophila RasGAP-related proteins

Number	RasGAP	GAP activity	LocusLink ID	Ensemble ID	Closest Drosophila gene	BLAST score	Class
1	RASA1	Yes	5921	ENSG00000145715	RasGAP	0.0	0
2	NF1	Yes	4763	ENSG00000005271	NF1	0.0	0
3	RASA2	Yes	5922	ENSG00000070069	Gap1	2e−95	1
4	GAP1IP4BP	Yes	22821	ENSG00000102637	Gap1	e−142	1
5	RASAL1	ND	8437	ENSG00000111344	Gap1	8e−57	1
6	CAPRI	Yes*	10156	ENSG00000105808	Gap1	8e−47	1
7	SYNGAP	Yes	8831	ENSG00000096170	CG5960	e−104	2
8	RASAL2	Yes*	9462	ENSG00000075391	CG5960	e−117	2
9	AF9Q34	ND	84635	ENSG00000136848	CG5960	e−115	2
10	FLJ21438	ND	64926	ENSG00000105122	CG5960	4e−48	2
11	IQGAP1	No	8826	ENSG00000140575	NF1	5e−14	3
12	IQGAP2	No	10788	ENSG00000132834	NF1	4e−15	3
13	IQGAP3*	ND	–	ENSG00000132721	NF1	9e−11	3
14	PLXNB2	?	23654	–	plexA	0.0	4

Human IQGAP1 and IQGAP2 lack obvious arginine fingers and in vitro GAP activity [92,93]. We propose the name IQGAP3 for the protein predicted by the *ENSG00000113271* gene. CAPRI showed Ca^{2+}-stimulated H-Ras GAP activity in vivo, but lacked detectable in vitro activity [256]. RasGAP activity for RASAL2 was inferred from its ability to complement a yeast Ira2 mutant [257]. Members of the plexin protein family include RasGAP-related domains. Only a single human and Drosophila plexin are included to make note of this fact. See Table 2 legend and the human and Drosophila GAP databases for more information.

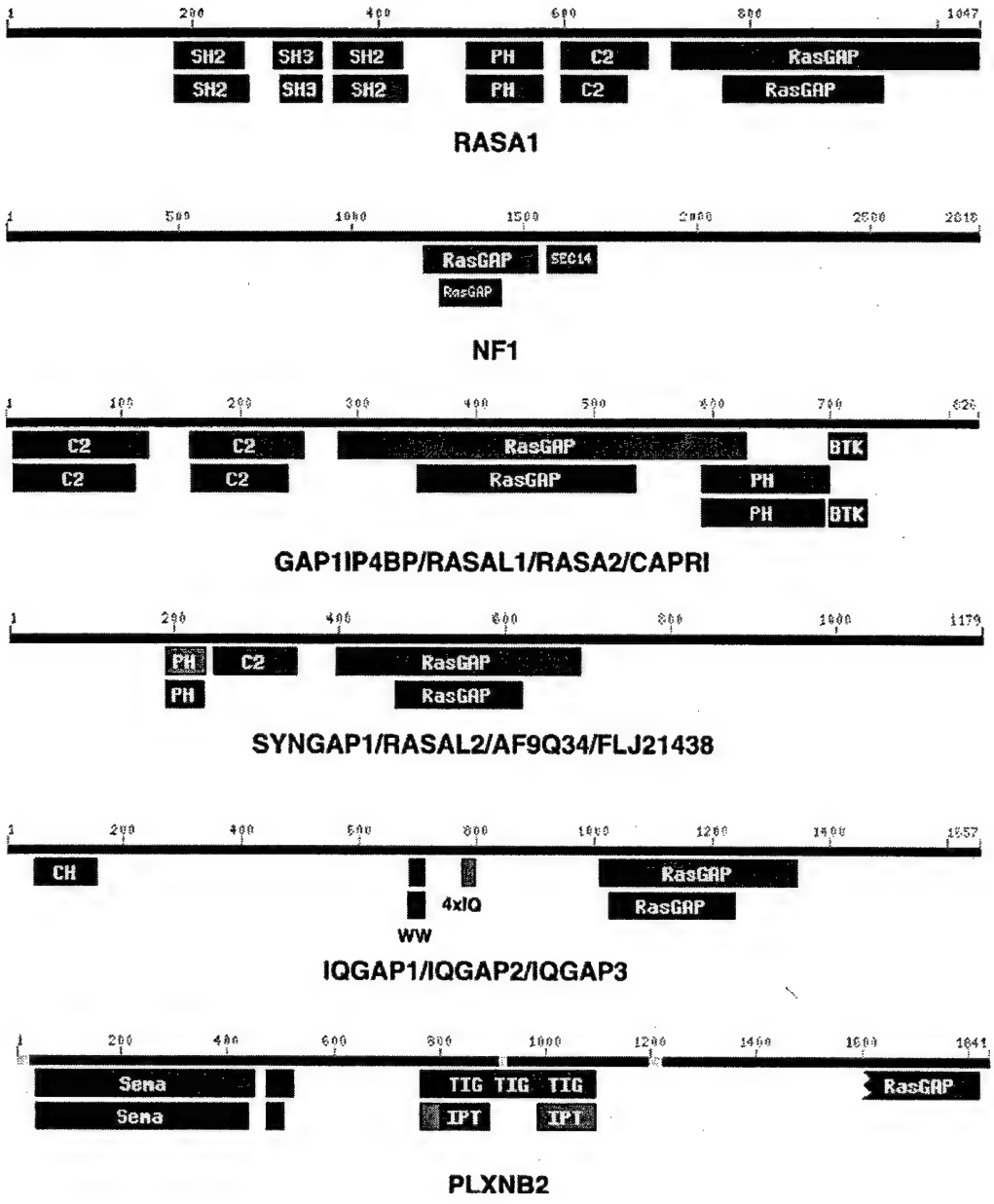

Fig. 9. Schematic structure of human RasGAP domain containing proteins. See Fig. 4 legend for details.

residues required for Ca^{2+} coordination, whereas the corresponding segments of Ca^{2+}-promoted Ras inactivator (CAPRI) resemble high affinity Ca^{2+}-binding domains. This may explain why Ca^{2+}-mobilization induced membrane translocation of CAPRI, but not of $Gap1^{m}$ or $GAP1^{IP4BP}$ [256]. Unlike CAPRI, other Gap1 family members interact with membranes via their PH domains. Thus, the constitutive plasma membrane association of $GAP1^{IP4BP}$ has been attributed to its PH domain serving as a high affinity PIP2 binding site [266]. By contrast, the PH domain of $Gap1^{m}$ binds PIP3 and has been implicated in the PI-3 kinase-dependent membrane translocation of $Gap1^{m}$ [267]. $GAP1^{m}$ also interacts with heterotrimeric G protein subunit $G\alpha12$ via its PH domain, and its GAP activity was stimulated by $G\alpha12$ [268]. In contrast to $Gap1^{m}$ and $Gap1^{IP4BP}$, a fusion protein representing the RasGAP domain of CAPRI lacked detectable in vitro H-Ras GAP activity [256]. However, Ca^{2+}-mobilization in transfected cells caused membrane translocation of CAPRI and a decrease in H-Ras-GTP, suggesting that the GAP activity of CAPRI is activated by calcium [256]. The fourth human Gap1-like protein, encoded by the *RASAL1* gene, has not been tested for GAP activity [269].

Whereas Ca^{2+} may activate CAPRI [256], the available evidence suggests that the GAP activity of rat SynGAP is inhibited upon phosphorylation by calmodulin-dependent

protein kinase II (CAMKII; Ref. [270]). SynGAP colocalized with N-methyl-D-aspartate (NMDA) glutamate receptors at excitatory synapses and its C terminus interacted with all three PDZ domains of PSD-95 [271]. A fusion protein representing the GAP domain of SynGAP stimulated H-Ras-GTP hydrolysis in vitro [271]. SynGAP was independently identified as a prominent CAMKII substrate in the postsynaptic protein complex, and RasGAP activity in this complex was inhibited either by an anti-SynGAP antibody or by activation of endogenous CAMKII. This suggested a model in which NMDA receptor-stimulated Ca^{2+} influx activates the Ras/MAPK cascade by inhibiting SynGAP activity [270]. Suggesting complex roles for Ras superfamily members at synapses, the guanylate kinase homology domain of PSD-95 also interacts with the SPAR RapGAP [245], and the PDZ domains of PSD-95 also serve as binding sites for the Rac1 exchange factor kalirin-7 [272]. Further complicating matters, alternative splicing generates at least four rat SynGAP isoforms with and without C-terminal PDZ domain binding motifs [270], and four human genes predict SynGAP-related proteins (Table 5, Fig. 9). The predicted *SYNGAP1*, *RASAL2*, and *AF9Q34* gene products include PH, C2, and RasGAP domains (Fig. 9). The fourth protein, FLJ21438, lacks the PH and C2 domains. However, a version of FLJ21438 that includes a partial PH domain is present in the Ensemble predicted protein database. A simpler situation exists in Drosophila, where only a single SynGAP-related protein is predicted by the *CG5960* gene.

Human IQGAP1 and IQGAP2 are characterized by the presence of an N-terminal calponin-homology F-actin binding domain, a centrally located WW domain, four calmodulin-binding IQ motifs, and a RasGAP-related C-terminal segment [93,273]. The RasGAP-related segments of IQGAPs lack obvious arginine fingers and rather than acting as GAPs for several tested GTPases, both IQGAPs inhibited the intrinsic and RhoGAP-stimulated activity of Rho family members Cdc42 and Rac1 [92,93]. The interaction between IQGAP1 and Cdc42 required the RasGAP domain and sequences on either side of this segment [92], and similar results were found for IQGAP2 [93]. Both IQGAPs have significant coiled-coil potential and purified IQGAP1 formed dimers and cross-linked actin microfilaments [274]. IQGAP1 may mediate cross-talk between Cdc42/Rac1 and calmodulin, since Ca^{2+}/calmodulin regulates the interaction between IQGAP1 and Cdc42 [275,276]. Endogenous IQGAP1 localizes to the Golgi in some cells [277] and to adherens junctions in others. The detection of a complex containing IQGAP1, E-cadherin, and β-catenin in immunoprecipitates, and the finding that overexpressed IQGAP1 displaced α-catenin from cell junctions were among results that suggested a role for IQGAP1 in the regulation of cadherin-based cell adhesion [278]. However, loss of murine IQGAP1 caused no defects beyond a modest increase in late onset gastric hyperplasia. This mild phenotype is unlikely due to functional redundancy with IQGAP2, since IQGAP1 and IQGAP2 have largely nonoverlapping expression patterns [279]. However, a third potential human IQGAP paralog with an unknown expression pattern was identified in this survey (Table 5). No obvious Drosophila IQGAP ortholog exists, which is remarkable since IQGAP-like proteins have been identified in many other phylogenetic groups, including budding and fission yeast, where they play essential roles in cytokinesis [280,281].

Semaphorins are a large family of proteins that are best known for mediating repulsive cell–cell interactions. Some semaphorins interact directly with plexin receptors, whereas others, such as semaphorin-3A, signal through receptors that consist of neuropilin ligand binding and plexin signal transducing subunits [282,283]. Ten mammalian plexins form four subfamilies: Plexin A1–4, Plexin B1–3, Plexin C1, and Plexin D1 [284,285]. Two Drosophila plexins (Plexin A and Plexin B) play roles in axon guidance [286,287]. Direct interactions between Rho family GTPases and plexins have been detected. Thus, two distinct segments of Drosophila Plexin B interact with activated Rac1 and RhoA, and dosage-sensitive genetic interactions suggest that Plexin B inhibits Rac signals by blocking its interaction with PAK, while enhancing RhoA output [287]. Two groups also reported interactions between mammalian Plexin B1 and activated Rac1, but not RhoA or Cdc42 [288,289], and Plexin A1 was found to interact with Rnd1 [290]. The cytoplasmic segment of plexins consists of two CDs that are required for semaphorin signaling [291]. The Rac1-binding site of mammalian Plexin B1 lies between these CDs and is distantly related to a Rho GTPase binding CRIB motif [289]. Interestingly, the two conserved plexin cytoplasmic segments resemble N-terminal and C-terminal segments of RasGAP catalytic domains, respectively, and mutations of Plexin-A1 arginines that correspond to RasGAP arginine finger residues resulted in mutant proteins that lacked the ability to stimulate semaphorin-3A-induced collapse of neuropilin-1 and plexin A1 cotransfected COS cells [290]. Thus, although the role of the RasGAP-related segments of plexins remains unclear, we included one human and one Drosophila plexin in Table 5 to make note of their RasGAP-relatedness.

16. Introduction to Rho GTPases

Approximately 12% of the 7800 papers identified in a PubMed "Ras GTPase" keyword search were published in 2001, whereas close to 28% of 2260 "Rho GTPase" papers were published in the same year. Rather than attempting to summarize this very active field, the following paragraph lists some of the most important research headlines and refers to reviews that can be consulted for more information.

Like the members of the Ras group, Rho GTPases largely transduce extracellular signals into intracellular responses. Best known among the latter are rearrangements of the F-actin cytoskeleton [292], but Rho family members also

control processes that do not obviously involve cytoskeletal changes, such as gene expression, or vesicular trafficking. A comprehensive review of the early literature on Rho regulators, effectors, and various Rho-controlled processes is available [293]. More recent reviews have focused on the role of Rho GTPases in development [294], in cell migration and tumor metastasis [295], in neuronal morphogenesis [296], on cross-talk between Rho and Ras family members [297], and on multiple levels at which the activity of Rho GTPases is controlled [298].

17. RhoGAP-related proteins

Remarkably, RhoGAP and RhoGEF domain-containing proteins constitute the 31st and 41st largest protein families in the human genome, respectively, with 60 RhoGAP- and 53 RhoGEF-related genes listed in the latest Ensemble release. In addition to the 60 Ensemble RhoGAP genes, this survey found another nine genes that predict RhoGAP domain containing proteins. Also included in Table 6 is the *BNIP2* gene, whose product stimulates Cdc42-GTP hydrolysis, but which lacks a typical RhoGAP domain. We also found 21 Drosophila genes that predict RhoGAP domain containing proteins. Observations that several RhoGAPs exhibit activity towards only some Rho GTPases whereas others associate with specific receptors may help to explain why it takes so many proteins to regulate a considerably smaller number of Rho family members.

The only protein in Table 6 that does not include a RhoGAP domain, BNIP2, was identified as one of three proteins that interacted with the adenovirus E1B 19K protein and with a related segment of Bcl-2 [299]. BNIP2 was subsequently found to be tyrosine phosphorylated by fibroblast growth factor receptor-1 [300]. The 314-residue BNIP2 protein includes a Sec14 domain (Fig. 10), and resembles the noncatalytic domain of Cdc42GAP (the *ARHGAP1* gene product; see Fig. 11). BNIP2 interacts with Cdc42GAP and Cdc42 in a phosphotyrosine-dependent manner, and modestly stimulated the release of radioactivity from [γ-32P]GTP-loaded Cdc42 [300]. Although other reasons besides GTP hydrolysis (i.e. nucleotide release) might explain this result, the same authors recently reported that specific arginine residue substitutions within the Sec14 domain reduce the GAP-like activity of BNIP2 [301]. We included BNIP2 in Table 6 to make note of these facts, but the BNIP2-related Drosophila *CG11593* gene product was not counted as a potential RhoGAP in Table 1.

Seven RhoGAP domain containing human proteins without closely related paralogs are listed as structural class 0 in Table 6. Five of these proteins, none of which includes other domains recognized in a CD search, lack closely related Drosophila orthologs. For one of these—KIAA1391—Locuslink lists three related loci, suggesting the existence of a protein family. However, one of these related loci appears to be KIAA1391 itself, the second (LOC135088) is only very distantly related to KIAA1391, and the KIAA1391-like protein segment predicted by the third is not currently predicted to include a RhoGAP domain. The remaining two proteins in this category, RalBP1 and KIAA1688, share similarity with a Drosophila protein that extends beyond their RhoGAP domains. Thus, KIAA1688 harbors a myosin tail homology-4 (MyTH4) domain upstream of a RhoGAP domain (Fig. 10). MyTH4 domains are found in the tails of several unconventional myosins and in a plant kinesin-like calmodulin binding protein, and a role in microtubule binding has been suggested [302]. The Drosophila *CG3421* gene predicts a protein that is highly related to KIAA1688. RalBP1 (also known as RLIP76 or RIP1) interacts with activated RalA and functions as a GAP for Cdc42 and Rac, but not Rho, thus providing a link between Ral and Cdc42/Rac [303–305]. RalBP1 interacts with two components of the endocytic machinery, AP2μ2 and POB1, suggesting a role in endocytosis [306,307]. The Drosophila Rlip protein is 40% identical to human RalBP1.

The remaining 62 human proteins in Table 6 fall into 21 structural classes (Figs. 10–12). The first of these consists of ABR and BCR. The *BCR* or breakpoint cluster region gene was identified as the fusion partner of the ABL tyrosine kinase in the Philadelphia chromosome translocation of chronic myelogenous leukemia [308]. ABR stands for active BCR-related gene [309], to distinguish it from three other BCR-related genes on human chromosome 22 which may not be transcribed [310,311]. The chromosome 22 *ENSG00000128226* gene, which predicts a protein that is virtually identical to the RhoGAP domain of BCR, is likely to be one of these pseudogenes and was not included in Table 6. The ABR and BCR proteins resemble the product of the Drosophila *EG:23E12.2* gene in that all three proteins include potential RhoGEF and RhoGAP catalytic domains (Fig. 10). Fusion proteins representing the ABR and BCR RhoGEF domains very modestly stimulated nucleotide exchange on Cdc42, RhoA, Rac1, and Rac2, whereas the RhoGAP domains enhanced GTP hydrolysis by Cdc42, Rac1, Rac2, but not by RhoA [100]. Remarkably, an N-terminal segment of BCR, which is not represented in ABR [312], exhibited protein serine/threonine kinase activity in the absence of any structural similarity to protein kinases [313]. Mouse *Abr* null mutants had no obvious phenotype [314], whereas loss of *Bcr* was associated with an enhanced respiratory burst in neutrophils [315]. Functional redundancy between these two mouse proteins is likely, since combined loss of Abr and Bcr resulted in abnormal astroglia function and perinatal lethality [314].

The human *CHN1* and *CHN2* genes encode α-chimerin (also known as *n*-chimerin) and β-chimerin, respectively [316,317]. The RacGAP activity of the former protein is stimulated by phorbol ester and inhibited by lysophosphatidic acid [316]. The Drosophila *CG3208* gene predicts a protein with a very similar structure (Fig. 10).

Table 6
Human and Drosophila RhoGAP-related proteins

Number	RhoGAP	GAP activity	LocusLink ID	Ensemble ID	Closest fly gene	BLAST score	Class
1	BNIP2	Yes	663	ENSG00000140299	CG11593	5e – 43	9
2	RALBP1	Yes	10928	ENSG00000017797	Rlip	3e – 48	0
3	KIAA1688	ND	80728	ENSG00000147799	CG3421	e – 104	0
4	KIAA0013	ND	9824	ENSG00000140231	CG6811	9e – 15	0
5	C5ORF5	ND	51306	ENSG00000031003	–	–	0
6	KIAA1391	ND	57569	ENSG00000137727	CG6811	2e – 15	0
7	LOC135088	ND	–	ENSG00000146437	CG6811	3e – 14	0
8	LOC118743	ND	–	ENSG00000148756	CG6477	6e – 28	0
9	ABR	Yes	29	ENSG00000056800	EG:23E12.2	e – 113	1
10	BCR	Yes	613	ENSG00000100235	EG:23E12.2	3e – 98	1
11	CHN1	Yes	1123	ENSG00000128656	CG3208	3e – 90	2
12	CHN2	Yes	1124	ENSG00000106069	CG3208	4e – 88	2
13	GRAF	Yes	23092	ENSG00000145819	CG8948	e – 117	3A
14	GRAF2	Yes	–	ENSG00000145400	CG8948	1e – 35	3A
15	OPHN1	Yes	4983	ENSG00000079482	CG8948	e – 106	3B
16	AK057372	ND	–	688152.14021	CG8948	7e – 88	3B
17	LOC129896	ND	–	–	CG8948	3e – 11	3C
18	KIAA0672	Yes	9912	ENSG00000006740	CG4755	3e – 47	4
19	SH3BP	Yes	23616	ENSG00000100092	CG4755	9e – 40	4
20	NADRIN	Yes	55114	ENSG00000122279	CG4755	2e – 43	4
21	ARHGAP5	Yes	394	ENSG00000100852	CG8240	e – 154	5
22	P190A	Yes	2909	507394.68855	CG8240	e – 148	5
23	–	ND	–	ENSG00000146646	CG8267	5e – 19	5
24	RACGAP1	Yes	29127	ENSG00000042272	acGAP	2e – 83	6
25	FKSG42	ND	83956	–	acGAP	8e – 69	6
26	LOC121702	ND	–	–	acGAP	6e – 47	6
27	CENTD1	ND	116984	ENSG00000047365	CG4937	9e – 33	7
28	CENTD2	ND	116985	ENSG00000110220	CG4937	6e – 33	7
29	FLJ21065	ND	64411	ENSG00000113548	CG4937	5e – 22	7
30	MYO9A	Yes	4649	ENSG00000066933	–	–	8
31	MYO9B	Yes	4650	ENSG00000099331	–	–	8
32	ARHGAP1	Yes	392	–	CG6811	1e – 64	9
33	ARHGAP8	ND	23779	ENSG00000128401	CG6811	7e – 57	9
34	DLC1	Yes	10395	ENSG00000147340	CG8480	e – 110	10
35	KIAA0189	ND	9754	ENSG00000130052	CG8480	1e – 96	10
36	LOC90627	ND	–	ENSG00000133121	CG8480	e – 106	10
37	ARHGAP4	Yes*	393	ENSG00000089820	CdGAPr	3e – 17	11
38	SRGAP1	Yes*	57522	ENSG00000079081	CdGAPr	9e – 16	11
39	SRGAP2	ND	23380	ENSG00000117283	CG4755	2e – 14	11
40	SRGAP3	ND	9901	ENSG00000144544	EG:23E12.2	4e – 16	11
41	–	ND	–	ENSG00000146131	CG1412	1e – 12	11
42	–	ND	–	ENSG00000145185	CG4755	2e – 10	11
43	PARG1	Yes	9411	ENSG00000137962	CG3208	2e – 22	12
44	LOC51291	ND	51291	ENSG00000089639	EG:23E12.2	9e – 20	12
45	KIAA0223	ND	23526	ENSG00000099815	CG3208	6e – 21	12
46	FLJ10312	ND	79822	ENSG00000088756	CG3208	2e – 11	13
47	MACGAP	ND	93663	ENSG00000146376	CG6811	2e – 08	13
48	dJ1100H13.4	ND	–	ENSG00000124143	CG1976	4e – 13	13
49	DKFZp564B1162	ND	83478	ENSG00000138639	CG3208	6e – 22	14
50	KIAA0053	ND	9938	ENSG00000115972	CG4755	2e – 17	14
51	LOC58504	ND	58504	ENSG00000148592	CG4755	3e – 22	14
52	PIK3R1	No	5295	ENSG00000145675	–	–	15
53	PIK3R2	ND	5296	ENSG00000105647	–	–	15
54	KIAA1424	ND	57584	ENSG00000107863	CG1412	1e – 41	16
55	KIAA1501	ND	57636	ENSG00000068703	CG1412	1e – 37	16
56	LOC94677	ND	–	613407.5052	CG1412	2e – 33	16
57	LOC94790	ND	–	ENSG00000140667	CG1412	5e – 30	16
58	OCRL	ND	4952	ENSG00000122126	EG:86E4.5	1e – 98	17
59	INPP5B	No	3633	ENSG00000134678	EG:86E4.5	e – 110	17
60	ARHGAP9	Yes	64333	ENSG00000123329	CdGAPr	6e – 19	18
61	FLJ10971	ND	55767	ENSG00000148518	CdGAPr	3e – 25	18
62	–	ND	–	ENSG00000141354	CdGAPr	2e – 12	18

Table 6 (*continued*)

Number	RhoGAP	GAP activity	LocusLink ID	Ensemble ID	Closest fly gene	BLAST score	Class
63	KIAA0712	ND	9743	ENSG00000134909	CdGAPr	1e – 73	19
64	DKFZP434A1010	ND	93092	ENSG00000004777	CdGAPr	e – 105	19
65	KIAA1204	Yes*	57514	ENSG00000031081	CdGAPr	2e – 48	19
66	–	ND	–	ENSG00000143218	CdGAPr	7e – 20	19
67	F02569_2	ND	85360	ENSG00000105137	CG1976	1e – 47	20
68	–	ND	84144	ENSG00000097096	CG1976	7e – 63	20
69	ARHGAP6	Yes	395	ENSG00000047648	CG1748	6e – 34	21
70	AK054620	ND	–	ENSG00000147256	CG1748	3e – 24	21

See Table 2 and the human and Drosophila GAP databases for additional information. Potential RhoGAPs predicted by the Drosophila *CG16980*, *CG7122*, *CG7481* and *Rotund* genes are not included, since they are not particularly closely related to any human RhoGAP. Yes* in the activity column means that RhoGAP activity was inferred from the absence of stress fibers in ARHGAP4 overexpressing fibroblasts or that GAP activity was detected for likely murine orthologs of srGAP1 and KIAA1204.

The human 'G protein regulator associated with focal adhesion kinase'(*GRAF*) gene product is the prototype of a class of RhoGAPs that also includes the proteins encoded by the *GRAF2*, oligophrenin-1 (*OPHN1*), and *AK057372* genes. All four proteins in this group harbor PH domains upstream of RhoGAP domains, but whereas GRAF and GRAF2 also include C-terminal SH3 domains, the other two do not. The protein predicted by the Drosophila *CG8948* gene is also predicted to lack a C-terminal SH3 domain. However, the Drosophila protein is most closely related to GRAF and GRAF2, and a translated BLAST search of the Drosophila genome finds a GRAF-like SH3 domain just downstream of the *CG8948* open reading frame. Recent entries in the LocusLink database are two "similar to GRAF" loci on chromosomes 14q11 and 22q11. Proteins predicted by these loci are most closely related to a recent GenBank entry, identified as "similar to oligophrenin". The latter protein is listed by its *LOC129897* gene name in Table 6 and includes a RhoGAP-like domain but no obvious PH or SH3 segments. Both of the "similar to GRAF" proteins include similarity to N-terminal and C-terminal segments of the LOC129897 protein, but neither protein at this time is predicted to include an obvious RhoGAP domain.

GRAF and GRAF2 have been implicated in integrin signaling. Thus, the SH3 domain of chicken GRAF interacted with a proline-rich segment of focal adhesion kinase FAK [102], whereas the SH3 domain of PSGAP (PH and SH3 domain containing RhoGAP; the likely mouse ortholog of GRAF2) interacted with a similar proline-rich segment of FAK-related PYK2 [318]. Both GRAF and PSGAP/GRAF2 exhibit GAP activity for Cdc42 and RhoA, but not for Rac1 [102,318,319]. Interestingly, although PSGAP interacts with both PYK2 and FAK, tyrosine phosphorylation of PSGAP was only observed in cells that express both PSGAP and PYK2. Suggesting that PYK2 phosphorylation activates Cdc42 by inhibiting PSGAP activity, pull-down experiments with the Cdc42-binding domain of PAK1 showed an increase in Cdc42-GTP in cells that coexpress PSGAP and PYK2 [318]. We also note that the SH3 domains of GRAF and GRAF2 have also been found to interact with Rho effector kinase PKNβ [319], and that the *OPHN1* gene harbors loss-of-function mutations in a subset of patients with X-linked mental retardation [19].

The proteins predicted by the human *SH3BP1*, *RICH1*, and *KIAA0672* genes most closely resemble the Drosophila *CG4755* gene product. All proteins in this group are of similar size and include central RhoGAP domains (Fig. 10). The 3BP-1 protein (encoded by the *SH3BP1* gene) was identified as an interactor with the SH3 domain of the ABL tyrosine kinase [320]. This protein has in vitro Rac GAP activity and inhibits Rac-dependent membrane ruffling [321]. Rat nadrin was first described as a neuronal protein that acted as a GAP for Cdc42, Rac1, and RhoA [322]. The likely human ortholog of this protein was found as a two-hybrid interactor with the SH3 domain of putative Cdc42 effector CIP4 [323]. The human protein was named RICH-1 for RhoGAP interacting with CIP4 homologs, and the name RICH-2 for the related KIAA0672 protein was proposed [323]. Like rat nadrin, human RICH-1 and RICH-2 stimulated the GTPase activity of Cdc42 and Rac1, but unlike nadrin neither of the human proteins showed activity towards RhoA [323]. Rather than a species difference, this likely reflects differences in the in vitro GAP assay conditions (for example, see Ref. [324]).

Three related RhoGAPs are predicted by the human *GRLF1*, *ARHGAP5*, and *ENSG00000146646* genes. The product of the first gene is usually referred to as p190 RhoGAP (*GRLF1* recalls the mistaken identification of p190 RhoGAP as a protein binding to the glucocorticoid receptor gene promoter). The related *GRLF1* and *ARHGAP5* gene products, hereafter referred to as p190A and p190B, include N-terminal GTP-binding segments followed by several FF domains [325], which in turn are followed by C-terminal RhoGAP domains (Fig. 10). The *ENSG00000146646* gene predicts a 170-residue RhoGAP domain segment that is 92% identical to human p190B and 55% identical to p190A. A 586-residue version of this protein is present in the Ensemble-predicted peptide database. Although this larger protein lacks the N-terminal GTP-binding segment, a search of the predicted protein set with this part of p190B identifies a 92% identical peptide encoded

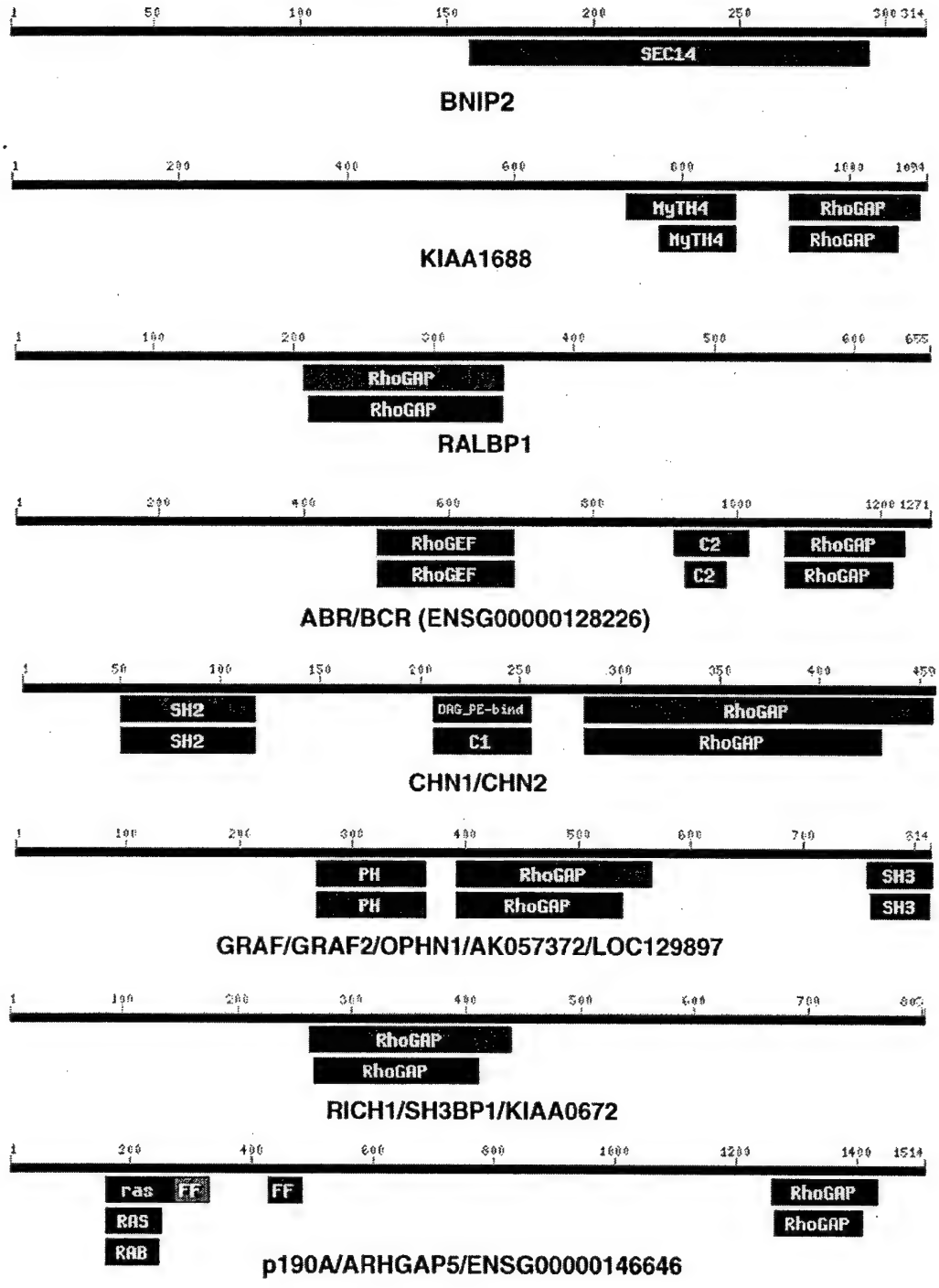

Fig. 10. Schematic structure of human RhoGAP domain containing proteins. See Fig. 4 legend for details.

on the same chromosome 7p11.2 contig. Thus, the human genome is likely to encode three p190 RhoGAP paralogs. The 1019-amino-acid Drosophila *CG8240* gene product is 32% identical over most of its length to p190A and p190B, but lacks a RhoGAP domain. However, the Drosophila protein that is most closely related to the RhoGAP domains of p190A or p190B is predicted by the *CG8267* gene, which maps immediately downstream of *CG8240* in the same transcriptional orientation. Thus, it appears likely that CG8240 and CG8267 together represent a single Drosophila p190-like gene. We have counted these two genes as one in Table 1.

First identified as a tyrosine phosphorylated protein that forms complex with p120 RasGAP in serum stimulated cells [252,326], the C-terminal catalytic domain of p190A exhibits preferential activity towards Rho [327]. Among other

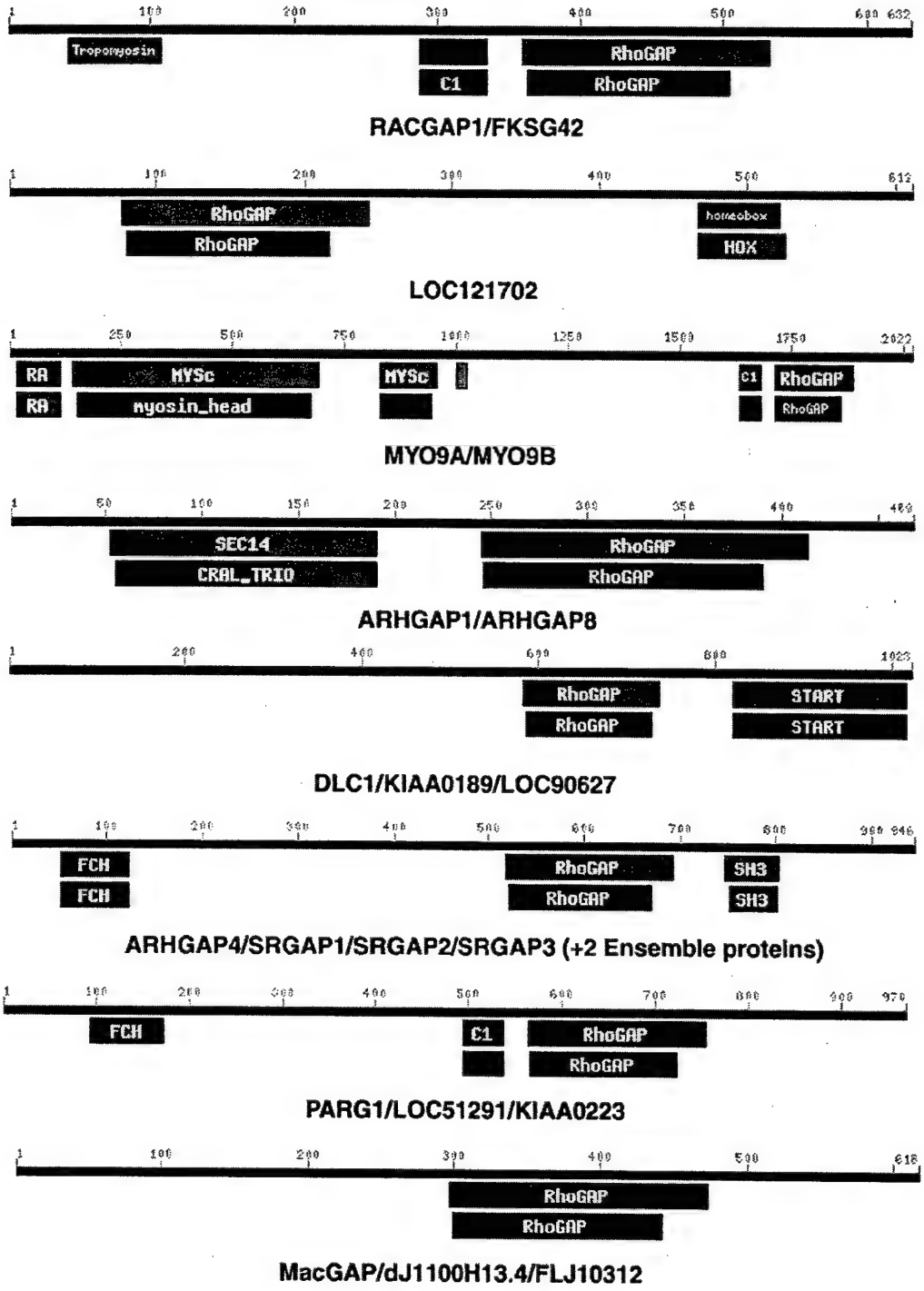

Fig. 11. Schematic structure of human RhoGAP domain containing proteins. See Fig. 4 legend for details.

findings, the Ras-related N-terminal segment of p190A binds GTP [328], and may regulate its RhoGAP activity [329]. Mice expressing an N-terminally truncated form of p190A without the GTP binding segment exhibit several neuronal defects [330], reflecting a role for p190A in axon guidance and fasciculation [331].

The proteins predicted by the human *RACGAP1* and *FKSG42* genes are 85% identical and have a similar domain structure (Fig. 11). Both proteins are most similar to Drosophila acGAP, and are more distantly related to the *rotund* RacGAP. It has been noted that Drosophila *rotund* and the *RACGAP1* gene product (known as mgcRacGAP

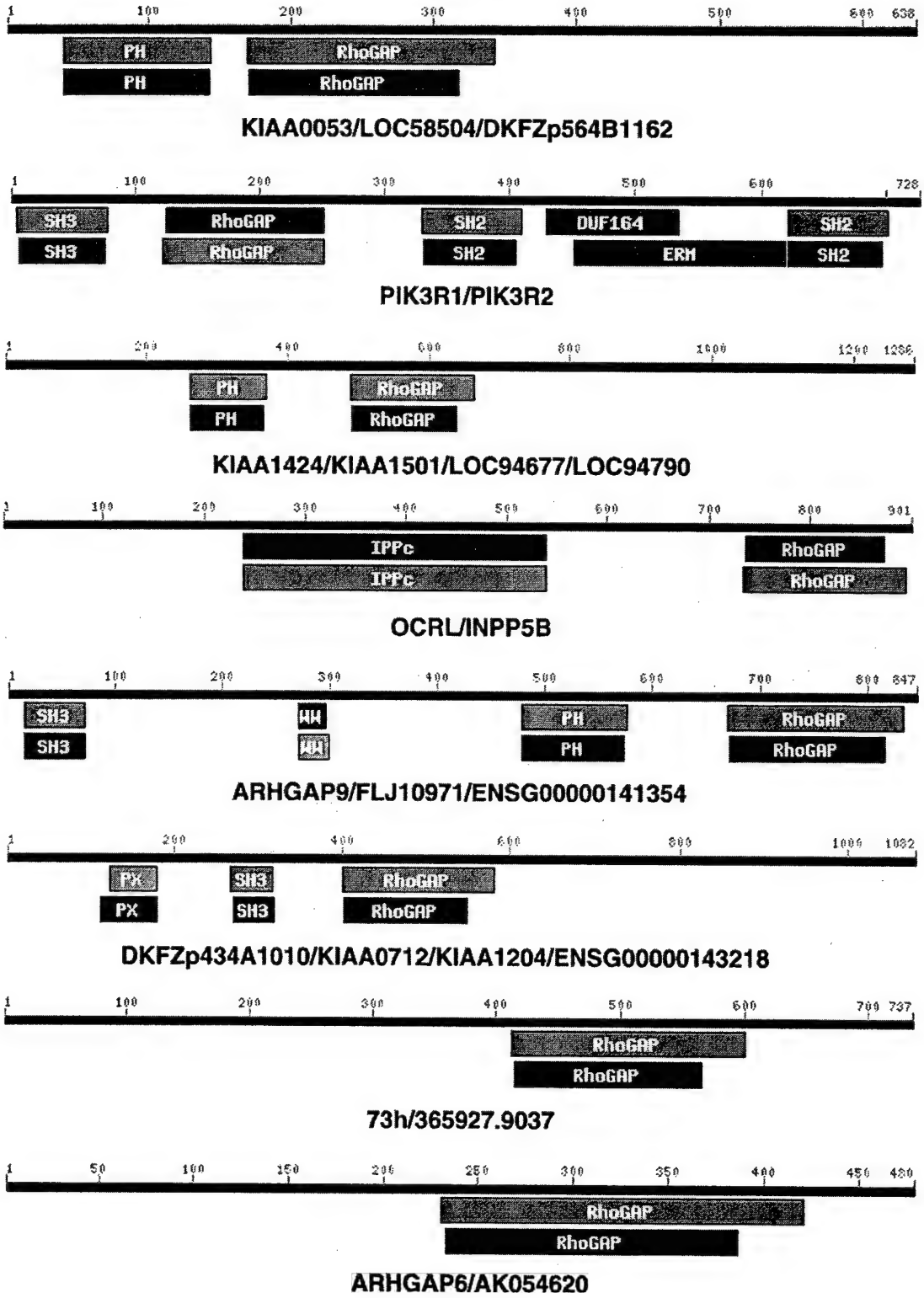

Fig. 12. Schematic structure of human RhoGAP domain containing proteins. See Fig. 4 legend for details.

for male germ cell RacGAP) are both expressed in male germ cells. Another similarity is that both proteins are GAPs for Rac and Cdc42, but not for Rho [332,333]. A novel GenBank entry (*LOC121702*; Fig. 11) includes an N-terminal RhoGAP domain that is almost identical to that of FKSG42 and a C-terminal homeobox domain. Whether this

unusual protein represents the product of a functional gene remains to be determined.

Three human centaurin delta paralogs include both ArfGAP and RhoGAP domains (see Section 8 and Fig. 4). No Drosophila centaurin delta orthologs exist, and the same is true for two differentially expressed unconventional human myosins encoded by the *MYO9A* and *MYO9B* genes (Fig. 11). Both genes were first identified in rats and predict proteins with N-terminal myosin head domains followed by six (MYO9A) or four (MYO9B) calmodulin-binding IQ motifs and C-terminal RhoGAP domains [334–336]. The myosin head domain is preceded by a segment that resembles a Ras association domain [337] (RA in Fig. 11), but neither protein showed evidence of binding Ras [336,338]. The RhoGAP domain of both proteins stimulates the GTPase of Rho but not Rac isoforms, and purified human myosin-IXB is a mechanochemically active motor protein [339].

The proteins predicted by the human *ARHGAP1* and *ARHGAP8* genes are 50% identical and harbor Sec14 putative lipid binding domains upstream of RhoGAP domains. The *ARHGAP1* gene product is variously known as RhoGAP, p50RhoGAP, or Cdc42GAP [340,341], whereas *ARHGAP8* was found by cDNA sequencing and has not yet been functionally analyzed. The 1.2.0 Ensemble release lists two genes (*ENSG00000077955* and *ENSG00000128401*) that map to the same chromosome 22 contig and that predict essentially identical ARHGAP8 proteins. Only one of these genes has been included in Table 6. Human ARHGAP1 and ARHGAP8 share around 35% sequence identity with the Drosophila CG6811 protein, overexpression of which results in complex neuronal abnormalities [342].

Proteins predicted by the human *DLC1* (deleted in liver cancer-1; human ortholog of rat p122 RhoGAP), *KIAA0189*, and *LOC90627*, and the Drosophila *CG8480* genes predict ~ 110–120-kDa proteins that harbor RhoGAP and START domains (Fig. 11). START domains were first described as lipid binding domains present in eukaryotic signaling proteins [343], but have since been found in a wider variety of both prokaryotic and eukaryotic proteins [344]. The only functionally analyzed member of this group is rat p122 RhoGAP, which represents the likely ortholog of the (unpublished) human DLC1 protein. Rat p122 RhoGAP is a GAP for Rho but not Rac, and stimulated the PIP2 hydrolyzing activity of phospholipase Cδ1 in vitro [345]. The combination of these activities is interesting, since several actin-regulating proteins such as α-actinin, cofilin, gelsolin, and profilin bind PIP2, and since Rho enhances the activity of the enzyme that synthesizes PIP2, phosphatidylinositol 4-phosphate 5-kinase [346].

The product of the X chromosome *ARHGAP4* gene, known as RhoGAP4 or p115 RhoGAP C1 [347], is the prototype of a group of at least four and potentially six related human proteins (Table 6). Members of this group include the *ARHGAP4, SRGAP1*, and *SRGAP2* gene products, which harbor N-terminal Fes/CIP4 homology (FCH) domains [348], in addition to RhoGAP and SH3 domains (Fig. 11). The SRGAP3 protein is over 60% identical to SRGAP1, but lacks an FCH domain. However, the current database version of SRGAP3 appears to be truncated since a search of the Ensemble-predicted protein set with the FCH domain of SRGAP1 found a 72% identical protein encoded on the SRGAP3 contig. The Ensemble 1.2.0 release also lists related *ENSG00000146131* and *ENSG00000145185* genes on chromosomes 6p21.2 and 4p15.1, respectively. The 686-residue protein predicted by the former gene is virtually identical to SRGAP1 except for one small divergent segment, whereas the 128-residue protein predicted by the latter gene is identical to a RhoGAP domain segment of SRGAP2. These genes may be real, or represent genome assembly errors or pseudogenes and are included in Table 6 for the sake of completion only.

Robo receptors and their secreted Slit ligands are best known for their roles in repulsive axon guidance, but Slit proteins also promote axon branching, repel migrating neurons, and inhibit leukocyte chemotaxis [349,350]. The cytoplasmic segments of Robo receptors include conserved CC0, CC1, CC2, and CC3 domains [351,352]. The Drosophila Abelson tyrosine kinase and its Enabled substrate play opposing roles in Robo signaling by interacting with the CC1 and CC2 domains [353]. Recently, the SH3 domains of mouse slit-robo GAP1 (srGAP1), srGAP2, and srGAP3 were found to interact with a proline-rich segment within the Robo1 CC3 motif [354]. Several findings suggest that this interaction is functionally important. Among these, srGAP1 was found to exhibit GAP activity for RhoA and Cdc42 but not Rac1, and Slit enhanced the interaction between srGAP1 and Robo1, resulting in Cdc42 inactivation. Moreover, GAP-deficient srGAP1 interfered with Slit-mediated repulsion, as did constitutively active Cdc42 [354]. While these findings suggest an important role for srGAP proteins in Robo-Slit function, the current Drosophila genome sequence does not predict any close srGAP orthologs (Table 6). Thus, either Drosophila Slit-Robo-mediated axon repulsion does not require srGAP-like proteins, or a Drosophila srGAP ortholog remains to be identified. We note that *C. elegans* F12F6.5 represents a likely ortholog of mammalian srGAPs.

Three more human RhoGAPs include N-terminal FCH domains. Unlike members of the previous group, the PARG1, LOC51291 (Gem-interacting protein), and KIAA0223 (minor histocompatibility antigen HA-1) proteins include C1 lipid binding domains and lack SH3 domains (Fig. 11). PARG1 is the only member of this group that has yet been functionally analyzed. It was identified as a 150-kDa RhoGAP whose C terminus interacted with the fourth PDZ domain of protein tyrosine phosphatase PTPL1 in vitro (the C terminus of KIAA0223 also resembles a consensus PDZ binding site). A PARG1 RhoGAP domain fusion protein strongly stimulated Rho-GTP hydrolysis and showed weak GAP activity towards either Rac or Cdc42

[355]. Again, no human protein in this group is especially closely related to any Drosophila RhoGAP.

Human FLJ10312, MacGAP, and dJ1100H13.4 are related RhoGAP domain-containing proteins that have not been functionally analyzed. No likely Drosophila ortholog is apparent for this group, and the same is true for the group consisting of the DKFZp564B1162, KIAA0053, and LOC58504 proteins (the GenBank LOC58504 entry represents a C-terminal segment of a larger Ensemble predicted protein; we used the Ensemble sequence to represent this locus). A CD search finds a C-terminal myosin-tail motif in the LOC58504 protein and an N-terminal SH2 domain in the KIAA0053 proteins only (Fig. 12). However, the C-terminal regions of all three proteins are related and an N-terminal SH2-like motif is apparent in the LOC58504 sequence.

The human PI 3-kinase p85α and p85β regulatory subunits include RhoGAP-like domains (Fig. 12). No similar RhoGAP domain-containing PI 3-kinase subunits exist in Drosophila or *C. elegans*. As noted in Section 4, p85α interacted with but did not stimulate the GTPase activity of Cdc42 and Rac.

It remains unclear how many *KIAA1424*- and *KIAA1501*-like genes exist in the human genome. Thus, LocusLink currently lists both genes and four related loci, and at least six potential members of this group are also apparent upon searching the Ensemble confirmed and predicted protein sets. However, several predicted proteins share >90% sequence identity, raising the suspicion that some may represent genome assembly errors. Thus, only four potential human genes are included in this group in Table 6, although others may exist.

The KIAA1424 and KIAA1501 proteins are ~45% identical and include PH domains upstream of RhoGAP domains (Fig. 12). The current version of KIAA1424 extends approximately 500 amino acids beyond the C terminus of KIAA1501, suggesting that KIAA1501 may be truncated. Among related Ensemble genes, several predict proteins of over 1700 amino acids in length. The Drosophila CG1412 protein is closest to either KIAA1424 or KIAA1501. The 2110-amino-acid predicted pCG1412 protein harbors a PDZ domain approximately 1000 residues upstream of a RhoGAP domain. Unlike KIAA1424 or KIAA1501, the 1742-residue protein predicted by the KIAA1424-like *LOC94677* gene also includes an N-terminal PDZ domain and was included in Table 6 to make note of this similarity. We also included the *LOC94790* gene, because its encoded protein only shares ~80% sequence identity with KIAA1501. None of the proteins in this group has been functionally analyzed, but a mutant *CG1412* allele is listed in FlyBase (http://flybase.bio.indiana.edu/).

Among human phosphatidylinositol polyphosphate 5-phosphatases, the *OCRL* and *INPP5B* gene products are unique in including C-terminal RhoGAP-like domains [356,357]. The OCRL protein is defective in oculocerebrorenal syndrome of Lowe [24]. The OCRL and INPP5B proteins are 51% identical over their entire length and analysis of knockout mice suggests they serve at least partially redundant functions [358]. The two human proteins in this group also share around 35% identity to large segments of the Drosophila *EG:86E4.5* gene product, which has a similar domain structure (Fig. 12). The significance of the RhoGAP-related segment in these proteins remains unclear, as INPP5B did not associate with or stimulate the activity of Cdc42, Rac1, RhoA, H-Ras, or Rab5B in vitro [96].

Among the related *ARHGAP9*, *FLJ10971*, and *ENSG00000141354* gene products, only the first has been functionally analyzed [359]. The ARHGAP9 protein includes an N-terminal SH3 domain, followed by WW, PH, and RhoGAP domains (Fig. 12). The 316-amino-acid FLJ10971 sequence represents only a RhoGAP domain but is likely to be incomplete, since it is 98% identical to part of a 847-residue *M. fascicularis* protein that includes all four domains. The protein predicted by the *ENSG00000141354* gene does not include an N-terminal SH3 domain, but may also be truncated. No obvious Drosophila ortholog for this group is apparent, suggesting a specialized function in mammals. The ARHGAP9 RhoGAP domain stimulated the activity of Cdc42 and Rac1, and showed less activity towards RhoA [359].

Mouse CdGAP is a 820-amino-acid protein with an N-terminal RhoGAP domain that is 72% identical to the first half of the 1445-amino-acid human KIAA1204 protein. Mouse CdGAP showed GAP activity towards Cdc42 and Rac, but not Rho [360]. The closest Drosophila protein, the 1843-amino-acid *CdGAPr* gene product, includes a large C-terminal extension like the human protein, but also has approximately 400 amino acids including an SH3 domain upstream of its RhoGAP domain. Up to four human genes predict proteins with KIAA1204-like RhoGAP domains (category 19 in Table 6), and at least one of these also includes an upstream SH3 domain. Thus, although current GenBank versions of the human *DKFZp434A1010* gene product (also known as N-chimerin homolog F25965_3) appear N-terminally truncated, a version in the Ensemble predicted protein database includes an upstream SH3 domain as well as a partially aligned Phox homology (PX) domain (Fig. 12). PX domains bind phosphoinositides and help target proteins to membranes [361]. Although its function in DKFZp4341010 remains to be established, the PX domain-like segment is conserved in Drosophila CdGAPr.

Two alternatively spliced RhoGAP domain containing proteins, FLJ13511and F02569_2, are produced by the human *73h* gene. Both proteins are about 80% identical to a larger protein predicted by a murine cDNA, suggesting that at least one of the human proteins is truncated. Although no closely related protein is present in the most recent GenBank or Ensemble confirmed protein databases, 1016-amino-acid Ensemble-predicted protein 365927.9037 produced an e – 114 BLAST score when compared to the mouse protein.

The prototype of the final group of human RhoGAPs is the protein predicted by the *ARHGAP6* gene, which shares approximately 41% sequence identity with the protein predicted by cDNA AK054620. The *ARHGAP6* gene maps to a 500-kb X chromosome segment deleted in patients with micropthalmia with linear skin defects syndrome [362], and the encoded protein acted as a GAP for RhoA, but not Rac1 or Cdc42 in vitro [101]. Gene-targeted mice expressing a truncated version of *Arhgap6* without the RhoGAP domain were normal in all respects [101].

18. Concluding remarks

The availability of draft and soon to be complete human and Drosophila genome sequences allows the assembly of parts list of functionally related and evolutionary conserved proteins such as the one presented here, and marks the end of the age of innocence for biologist. Thus, conclusions of many biochemical and overexpression studies are seen in a different light when realizing that around 0.5% of human and Drosophila genes may encode GAPs for Ras superfamily members, many of which have not been analyzed. Obviously, while reductionist studies of individual proteins have contributed greatly to our current understanding of this protein family, additional studies in organisms amenable to genetic analysis will undoubtedly have an important role to play in the future. The survey presented here should make it easier to design such studies.

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